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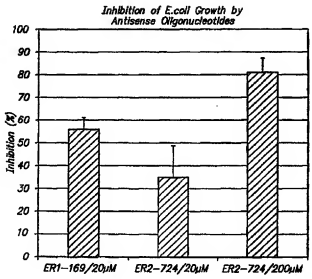

(57) Abstract

The invention relates to antisense oligonucleotides which modulate the expression of the ribonucleotide reductase or the secA genes in microorganisms. This invention is also related to methods of using such oligonucleotides in inhibiting the growth of microorganisms. These antisense oligonucleotides are particularly useful in treating pathological conditions in mammals which are mediated by the growth of microorganisms.

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ANTISENSE OLIGONUCLEOTIDE SEQUENCES AS INHIBITORS OF MICROORGANISMS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to antisense oligonucleotides which modulate the activity of the ribonucleotide reductase genes and the secA genes in microorganisms. This invention is also related to methods of using such compounds in inhibiting the growth of microorganisms.

These antisense oligonucleotides are particularly useful in treating pathological conditions in mammals which are mediated by the growth of microorganisms. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

These antisense oligonucleotides may also be used as anti-microbial agents for agricultural applications such as crop protection.

References

The following publications, patent applications and patents are cited in this application as superscript numbers:

1. Nordlund and Eklund "Structure and function of the *Escherichia coli* ribonucleotide reductase protein R2", *J. Mol. Biol.* (1993) **232**:123-164;

2. Carlson et al., "Primary structure of the *Escherichia coli* ribonucleoside diphosphate reductase operon", *PNAS* USA (1984) **81**:4294-4297;

3. Nilsson et al., "Nucleotide sequence of the gene coding for the large subunit of ribonucleotide reductase of *Escherichia coli* Correction", *Nucleic Acids Research* (1988) **16**:4174;

4. P. Reichard, "The anaerobic ribonucleotide reductase from *Escherichia coli*", *J. Biol. Chem.* (1993) **268**:8383-8386;

5. Nordlund et al., *Nature* (1990) **345**:593-598;

6. der Blaauwen et al., "Inhibition of preprotein translocation and reversion of the membrane inserted state of secA by a carboxyl terminus binding Mab", *Biochemistry* (1997) **36**:9159-9168;

7. McNicholas et al., "Dual regulation of Escherichia coli secA translation by distinct upstream elements", *J. Mol. Biol.* (1997) **265**:128-141;

8. U.S. Patent No. 5,294,533;

9. Gasparro et al., "Photoactivatable antisense DNA: Suppression of ampicillin resistance in normally resistant *Escherichia coli", Antisense Research and Development* (1991) **1**:117-140;

10. White et al., "Inhibition of the multiple antibiotic resistance (mar) operon in Escherichia coli by antisense DNA analogs", *Antimicrobial Agents and Chemotherapy* (1997) **41**:2699-2704;

11. Nielsen et al., *Science* (1991) **354**:1497;

12. Good and Nielsen, "Inhibition of translation and bacterial growth by peptide nucleic acid targeted to ribosomal RNA", *PNAS USA* (1998) **95**:2073-2076;

13. Buchardt, deceased, et al., U.S. Patent No. 5,766,855;

14. Buchardt, deceased, et al., U.S. Patent No. 5,719,262;

15. U.S. Patent No. 5,034,506;

16. Altschul, et al.,"Basic local alignment search tool", *J. Mol. Biol.* (1990) **215**:403-10;

17. Devereux. et al., "A comprehensive set of sequence analysis programs for the VAX", *Nucleic Acids Res.* (1984) **12**:387-395;

18. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1989, 1992);

19. Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore Maryland (1989);

20. Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor MI (1995);

21. Vega et al., *Gene Targeting*, CRC Press, Ann Arbor MI (1995);

22. *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988)

23. U.S. Patent 5,023,252, issued June 11, 1991

24. Felgner et al., U.S. Patent No. 5,580,859.

25. U.S. Patent 5,011,472

26. *Remington's Pharmaceutical Sciences*, Mace Publishing Company, Philadelphia PA 17th ed. (1985);

27. Perbal, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, New York (1988).

28. *PCR Protocols: A Guide To Methods And Applications*, Academic Press, San Diego, CA (1990).

29. Dower, W.J., *Nucleic Acids Res.* (1988) **16**:6127;

30. Neuman et al., *EMBO J.* (1982) **1**:841;

31. Taketo A., *Biochim Biophys. Acta* (1988) **949**:318;

32. Miller J.H. *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1972);

33. Horwitz J.P., *J. Med. Chem.* (1964) 7:574;

34. Mann et al., *Biochem.*(1991) **30**:1939;

35. Olsvik, et al., *Acta Pathol. Microbiol. Immunol. Scand. [B]* (1982) **90**:319;

36. Laemmli, U.K., *Nature* (1970) **227**:680;

37. Choy et al., *Cancer Res.*(1988) **48**:2029;

38. Wright and Anazodo, *Cancer J.* (1988) 8:185-189;

39. Chan et al., *Biochemistry* (1993) 32:12835-12840;

40. Carpentier P.L., *Microbiology 4th ed.* W.B.Saunders Company (1977); and

41. Wright et al., *Adv. Enzyme Regul.* (1981) 19:105-127.

All of the above publications, patent applications and patents are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

Ribonucleotide reductase catalyzes the *de novo* production of deoxyribonucleotides. The enzyme reduces the four main ribonucleotides to the corresponding deoxyribonucleotides required for DNA synthesis and repair (Wright et al.[41]).

In mammalian and bacterial cells, *de novo* production of deoxyribonucleotides by ribonucleotide reductase is usually highly regulated on different levels in order to produce the correct amount of deoxyribonucleotides for DNA synthesis. In the DNA viruses, the metabolism of the host cell is directed towards production of viral DNA by virus encoded ribonucleotide reductases (Nordlund and Eklund[1]).

Mammalian cells and many DNA viruses and prokaryotes, have a heterodimeric iron-containing ribonucleotide reductase enzyme of the $\alpha_2\beta_2$ type. For example, ribonucleotide reductase from *E. coli* is a multi-subunit $\alpha_2\beta_2$ enzyme where the two homo-dimeric proteins are denoted R1 and R2. The larger α_2 protein, R1, contains the binding sites for substrate and allosteric effectors and also the redox-active cysteine residues. Protein R1 has a molecular mass of 2 x 86,000 where each subunit contains 761 residues. The smaller β_2 protein, denoted R2, contains the dinuclear ferric center and a stable free tyrosyl radical necessary for the enzymatic activity. The R2 protein has a molecular mass of 2 x 43,500, where each subunit contains 375 amino acid residues (Nordlund and Eklund[1]).

The nucleotide sequence of the *E. coli* K-12 DNA comprising the operon for the structural genes of the subunits of ribonucleotide reductase has been determined. The DNA sequence includes a total length of 8557 nucleotides. An open reading frame

between nucleotides 3506 and 5834 has been identified as the nrdA gene. An open reading frame between nucleotides 6012 and 7139 encoding a 375-amino acid polypeptide has been identified as the nrdB gene (Carlson et al.[2], and Nilsson et al.[3]). The sequences of the nrdA and nrdB genes for *E. coli* are shown in Figures 1 and 2.

In *E. coli*, the synthesis of ribonucleotide reductase is controlled at the level of transcription. The nrdA and nrdB genes direct the synthesis of a 3.2 kilobase polycistronic mRNA. Perturbations in DNA replication, either a shift up in growth conditions or an inhibition of DNA synthesis leads to increased synthesis of nrd mRNA (Carlson et al.[2]).

A separate anaerobic ribonucleotide reductase has also been identified from *E.coli*. The anaerobic *E. coli* reductase has a molecular mass of 145 kD and is a homodimer. The gene for the anaerobic reductase (nrdD) has been cloned and sequenced (P. Reichard[4]).

The ribonucleotide reductase R2 genomic or cDNA sequences are known for several other species such as bacteriophage T4, clam, mouse, *Saccharomyces cerevisiae*, vaccinia, herpes simplex virus types 1 and 2, varicella and Epstein-Barr virus (Nordlund et al.[5]). The sequence of the nrdE and nrdF which code for the ribonucleotide reductase genes of *S. typhimurium* are shown in Figure 3. The sequence of the ribonucleotide reductase gene of *Lactococcus lactis* is shown in Figure 4.

The secA gene of *E. coli* encodes for one component of a multi-component system for the secretion of proteins across the inner membrane of *E. coli* (der Blaauwen et al.[6]). The complete system consists of the SecB protein, a cytosolic chaperone, the SecA protein, the translocation ATPase and the heterotrimeric integral membrane SecY/SecE/SecG complex, which along with SecA serves as the preprotein channel. SecA protein plays a central role in the secretion process by binding the preprotein, secB protein, anionic phospholipids and SecY/SecE/SecG protein. These interactions allow SecA to recognize soluble preprotein and recruit it to translocation sites in the inner membrane. Once such protein translocation complexes have assembled; further steps require an ATP-driven cycle of insertion and de-insertion of

secA protein with the inner membrane, where each cycle appears to be coupled to the translocation of a segment of the preprotein.

SecA is the only component of the secretion apparatus that has been shown to be regulated. SecA is the second gene in the geneX-secA operon and its translation varies over a tenfold range depending on the status of protein secretion in the cell. During protein-export proficient conditions secA auto-represses its translation by binding to a site that overlaps the secA ribosome-binding site of genes-secA RNA. SecA protein can also dissociate a preformed 30 S-$tRNA^{MET}$-genes-secA RNA ternary complex in vitro. However, during a protein export block secA translation increases substantially although the mechanism responsible for this regulatory response has not been elucidated (McNicholas et al.[7]). The sequence of the secA gene of *E. coli* is shown in Figure 5.

The secA gene sequence has been identified for a number of other species including *Mycobacterium bovis* (Figure 6), *Mycobacterium tuberculosis* (Figure 7), *Staphylococcus aureus* (Figure 8), *Staphylococcus carnosus* (Figure 9), *Bacillus subtilis*, *Bacillus firmus*, *Listeria monocytogenes*, *Mycobacterium smegmatis*, *Borrelia burgdorferi*, *P. sativum*, *S. griseus*, and *Synechoccus sp.*

Antibiotics are important pharmaceuticals for the treatment of infectious diseases in a variety of animals including man. The tremendous utility and efficacy of antibiotics results from the interruption of bacterial (prokaryotic) cell growth with minimal damage or side effects to the eukaryotic host harboring the pathogenic organisms. In general, antibiotics destroy bacteria by interfering with the DNA replication, DNA to RNA transcription, translation (that is RNA to protein) or cell wall synthesis.

Although bacterial antibiotic resistance has been recognized since the advent of antimicrobial agents, the consequence of the emergence of resistant microorganisms, such resistance was historically controlled by the continued availability of effective alternative drugs. Now, drug resistance has emerged as a serious medical problem in the community, leading to increasing morbidity and mortality. The problem is worsened by the growing number of pathogens resistant to multiple, structurally

unrelated drugs. The situation has become so desperate that antibiotics once removed from use because of toxic effects may be prescribed in an attempt to deal with the otherwise untreatable drug resistant bacteria.

Antisense oligonucleotides have been used to decrease the expression of specific genes by inhibiting transcription or translation of the desired gene and thereby achieving a phenotypic effect based upon the expression of that gene (Wright and Anazado[38]). For example, antisense RNA is important in plasmid DNA copy number control, in development of bacteriophage P22. Antisense RNA's have been used experimentally to specifically inhibit *in vitro* translation of mRNA coding specifically from Drosophila hsp23, to inhibit Rous sarcoma virus replication and to inhibit 3T3 cell proliferation when directed toward the oncogene c-fos. Furthermore, it is not necessary to use the entire antisense mRNA since a short antisense oligonucleotide can inhibit gene expression. This is seen in the inhibition of chloramphenicol acetyltransferase gene expression and in the inhibition of specific antiviral activity to vesicular stomatitus virus by inhibiting the N-protein initiation site. Antisense oligonucleotides directed to the macromolecular synthesis operon of bacteria, containing the rpsU gene, the rpoD gene and the dnaG gene have been used for the detection of bacteria. (U.S. Patent No. 5,294,533[8]). Furthermore, photoactivatable antisense DNA complementary to a segment of the β-lactamase gene has been used to suppress ampicillin resistance in normally resistant *E. coli* (Gasparro et al.[9]). Antisense DNA analogs have also been used to inhibit the multiple antibiotic resistant (mar) operon in *Escherichia coli* (White et al.[10]).

Accordingly, there is a need to develop antisense oligonucleotides which will act to inhibit the growth of microorganisms.

SUMMARY OF THE INVENTION

This invention is directed to antisense oligonucleotides which modulate the expression of the ribonucleotide reductase and secA genes in microorganisms and pharmaceutical compositions comprising such antisense oligonucleotides. This

invention is also related to methods of using such antisense oligonucleotides for inhibiting the growth of microorganisms.

Accordingly, in one of its composition aspects, this invention is directed to an antisense oligonucleotide, which oligonucleotide is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism. The antisense oligonucleotide may have one or more phosphorothioate internucleotide linkages.

In another of its composition aspects, this invention is directed to an antisense oligonucleotide comprising from about 3 to about 50 nucleotides which is capable of binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an antisense oligonucleotide, which oligonucleotide is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism. The oligonucleotide may be modified, for example, the oligonucleotide may have one or more phosphorothioate internucleotide linkages.

In one of its method aspects, this invention is directed to a method for inhibiting the expression of the ribonucleotide reductase gene in a microorganism having a ribonucleotide reductase gene comprising, administering to said microorganism or to a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary

to the ribonucleotide reductase gene of the microorganism under conditions such that the expression of the ribonucleotide reductase gene is inhibited.

In another of its method aspects, this invention is directed to a method for inhibiting the expression of the secA gene in a microorganism having a secA gene, comprising administering to said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to the secA gene of the microorganism under conditions such that expression of the secA gene is inhibited.

In one of its method aspects, this invention is directed to a method for inhibiting the growth of a microorganism encoding a ribonucleotide reductase gene or a secA gene, which method comprises administering to said microorganism or a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited. Preferably, the antisense oligonucleotide is selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

In another of its method aspects, this invention is directed to a method for treating a mammalian pathologic condition mediated by a microorganism, which method comprises identifying a mammal having a pathologic condition mediated by a microorganism having a ribonucleotide reductase gene or a secA gene and administering to said mammal an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the sequence of the *E. coli* nrdA gene encoding the ribonucleotide reductase R1 subunit [SEQ ID NO:1].

Figure 2 is the sequence of the *E. coli* nrdB gene encoding the ribonucleotide reductase R2 subunit [SEQ ID NO:2]. The nrdB gene is encoded by nucleotides 7668 to 8798 of SEQ ID NO:2.

Figure 3 is the sequence of the *S. typhimurium* nrdE and nrdF genes encoding the ribonucleotide reductase subunits [SEQ ID NO:3]. The nrdE gene is encoded by nucleotides 836 to 2980 and the nrdF gene is encoded by nucleotides 2991 to 3950 of SEQ ID NO:3.

Figure 4 is the sequence of the *Lactococcus lactis* nrdEF operon encoding ribonucleotide reductase [SEQ ID NO:4].

Figure 5 is the sequence of the *E. coli* secA gene [SEQ ID NO:5].

Figure 6 is the sequence of the Mycobacterium bovis secA gene [SEQ ID NO:6].

Figure 7 is the sequence of the *Mycobacterium tuberculosis* secA gene [SEQ ID NO:7].

Figure 8 is the sequence of the *Staphylococcus aureus* secA gene [SEQ ID NO:8].

Figure 9 is the sequence of the *Staphylococcus carnosus* secA gene [SEQ ID NO:9].

Figure 10 is the sequence of the bovine herpes virus ribonucleotide reductase small subunit gene [SEQ ID NO:10].

Figure 11 is the sequence of the Herpes simplex virus type 1 UL39 gene encoding ribonucleotide reductase 1 [SEQ ID NO:11].

Figure 12 is the sequence of the Herpes simplex type 2 ribonucleotide reductase gene [SEQ ID NO:12]. The ribonucleotide reductase gene is encoded by nucleotides 419 to 3853 of SEQ ID NO:12.

Figure 13 is the sequence of the equine herpes virus 4 ribonucleotide reductase large subunit and small subunit [SEQ ID NO:13]. The large subunit is encoded by

nucleotides 77 to 2446 and the small subunit by nucleotides 2485-3447 of SEQ ID NO:13.

Figure 14 is a photograph of a Western blot of a polyacrylamide gel of the cellular protein from *E. coli* cells carrying a plasmid containing the mouse ribonucleotide reductase R2 gene after treatment with either 20μM or 200 μM of oligonucleotide AS-II-626-20.

Figure 15 is a graph of the inhibition of *E. coli* growth after treatment of *E. coli* cells with ribonculeotide reductase antisense oligonucleotides.

Figure 16 is a graph of the number of colony forming units/ml of *E. coli* cells after treatment with ribonucleotide reductase antisense oligonucleotides.

Figure 17 is a photograph of a Western blot of a polyacrylamide gel of cellular protein from *E. coli* cells after treatment with secA antisense oligonucleotides.

Figures 18a and 18b are graphs of the number of colony forming units/ml of *E. coli* cells after treatment with secA antisense oligonucleotides.

Figures 19a-g are graphs of growth curves of *E. coli* K12 after treatment with antisense oligonucleotides. Figure 19a shows the growth after treatment with 16 μM or 80 μM of antisense ES799 [SEQ ID NO:195]. Figure 19b shows the growth after treatment with 20 μM of antisense ES1739 [SEQ ID NO:229]. Figure 19c shows the growth after treatment with 80 μM of antisense ES851 [SEQ ID NO:197]. Figure 19d shows the growth after treatment with 80 μM of antisense ES553 [SEQ ID NO:188]. Figure 19e shows the growth after treatment with 80 μM of antisense ES646 [SEQ ID NO:191]. Figure 19f shows the growth after treatment with 80 μM of antisense ES1845 [SEQ ID NO:235]. Figure 19g shows the growth after treatment with 80 μM of antisense ES2537 [SEQ ID NO:254].

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds that inhibit the growth of microbes by inhibiting the expression of a ribonucleotide reductase protein or the secA protein. Without being limited to any theory, the compounds inhibit the expression of the ribonucleotide reductase or the secA protein by inhibiting the transcription of the gene

or the translation of the mRNA to protein. Such compounds include antisense oligonucleotides.

Definitions:

As used herein, the following terms have the following meanings:

The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complementary to the mRNA for the desired gene. Preferably, the antisense oligonucleotide is complementary to the mRNA for ribonucleotide reductase or secA.

The term "oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and inter-sugar (backbone) linkages. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted oligomers may be preferred over naturally occurring forms because of the properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes chimeric oligonucleotides which contain two or more chemically distinct regions. For example, chimeric oligonucleotides may contain at least one region of modified nucleotides that confer beneficial properties (e.g. increased nuclease resistance, increased uptake into cells) or two or more oligonucleotides of the invention may be joined to form a chimeric oligonucleotide.

The antisense oligonucleotides of the present invention may be ribonucleic or deoxyribonucleic acids and may contain naturally occurring or synthetic monomeric bases, including adenine, guanine, cytosine, thymine and uracil. The oligonucleotides may also contain modified bases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thioalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza

uracils, thymidines, cytosines or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

The antisense oligonucleotides of the invention may also comprise modified phosphorus oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatom or heterocyclic intersugar linkages. For example, the antisense oligonucleotides may contain methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. In one embodiment of the invention, the antisense oligonucleotides comprise phosphorothioate bonds linking between the four to six 3'-terminus nucleotides. In another embodiment, the phosphorothioate bonds link all the nucleotides. The antisense oligonucleotides may also have sugar mimetics.

The antisense oligonucleotides of the invention may also comprise nucleotide analogues wherein the structure of the nucleotide is fundamentally altered. An example of such an oligonucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in DNA (or RNA) is replaced with a polyamide backbone which is similar to that found in peptides (Nielsen et al.[11]; Good and Nielsen[12]; Buchardt, deceased, et al.[13], U.S. Patent No. 5,766,855; Buchardt, deceased, et al.[14], U.S. Patent No. 5,719,262). PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind more strongly to a complementary DNA sequence than to a naturally occurring nucleic acid molecule due to the lack of charge repulsion between the PNA strand and the DNA strand.

The oligonucleotides of the present invention may also include other nucleotides comprising polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may comprise morpholino backbone structures (U.S. Patent No. 5,034,506[15]).

The oligonucleotides of the present invention are "nuclease resistant" when they have either been modified such that they are not susceptible to degradation by DNA and RNA nucleases or alternatively they have been placed in a delivery vehicle which in itself protects the oligonucleotide from DNA or RNA nucleases. Nuclease resistant

oligonucleotides include, for example, methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. Suitable delivery vehicles for conferring nuclease resistance include, for example liposomes.

The oligonucleotides of the present invention may also contain groups, such as groups for improving the pharmacokinetic properties of an oligonucleotides, or groups for improving the pharmacodynamic properties of an oligonucleotide. Preferably, the oligonucleotides do not contain reporter groups or labels, such as fluorescent dyes or radioactive labels.

The antisense oligonucleotides may be complementary to the complete ribonucleotide reductase or secA gene including the introns. Preferably, the antisense oligonucleotides are complimentary to the mRNA region from the ribonucleotide reductase gene or the secA gene.

The antisense oligonucleotides may be selected from the sequence complementary to the ribonucletide reductase or secA genes such that the sequence exhibits the least likelihood of showing duplex formation, hair-pin formation, and homooligomer/sequence repeats but has a high to moderate potential to bind to the ribonucleotides reductase gene or the secA gene sequence and contains a GC clamp. These properties may be determined using the computer modeling program OLIGO Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN). This computer program allows the determination of a qualitative estimation of these five parameters.

Alternatively, the antisense oligonucleotides may also be selected on the basis that the sequence is highly conserved for either the ribonucleotide reductase or the secA genes between two or more microbial species. These properties may be determined using the BLASTN program (Altschul, et al.[16]) of the University of Wisconsin Computer group (GCG) software (Devereux J. et al.[17]) with the National Center for Biotechnology Information (NCBI) databases.

The antisense oligonucleotides generally comprise from at least about 3 nucleotides or nucleotide analogs, preferably from about 3 to about 50 nucleotides or

nucleotide analogs, more preferably, from about 7 to about 35 nucleotides or nucleotide analogs, most preferably from about 15 to about 25 nucleotide or nucleotide analogs.

Preferably, the antisense oligonucleotides comprise from 3 to about 50 nucleotides or nucleotide analogs, more preferably from 20 to about 50 nucleotides or nucleotide analogs and further comprise all or part of the sequences set forth in Tables 1, 2, 3, and 4 (below). Preferably, the oligonucleotides complementary to the ribonucleotide reductase gene comprise SEQ ID NOS.: 14 to 157 as shown in Tables 1 and 2 . Preferably, the antisense oligonucleotides complementary to the secA gene comprise the SEQ ID NOS.: 158 to 265 as shown in Tables 3 and 4.

Table 1
Antisense oligonucleotides that target the *Escherichia coli* K12 ribonucleotide reductase large subunit (R1)

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
14	ER1-16	CCGTCGCGCTTTGTCACCAG	61.1	-43.0
15	ER1-24	CTGTGCTACCGTCGCGCTTT	57.8	-42.0
16	ER1-33	TGATGCGCTCTGTGCTACCG	57.2	-40.2
17	ER1-44	TTTGTCGAGATTGAT GCGCT	53.3	-38.7
18	ER1-58	AGAACGCGATGGATTTTGTC	51.7	-38.4
19	ER1-71	TGCCGCCCAATCCAGAACGC	64.6	-46.0
20	ER1-79	AGTCCTTCTGCCGCCCAATC	57.7	-42.2
21	ER1-128	AAACTGAATGTGGGAGCGCA	55.5	-39.8
22	ER1-169	ATAATGGTTTCGTGGATGTC	55.5	-35.4
23	ER1-180	CGGCAGCCTTGATAATGGTT	54.2	-40.6
24	ER1-218	ATACTGATAATCCGGCGCAT	51.4	-39.4
25	ER1-252	TACGCAGGTGGAAGATCGCC	57.3	-41.4

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
26	ER1-294	GGTCGTACAGCGCAGGCGGC	64.4	-45.9
27	ER1-320	GCCCATCTCGACCATTTTCA	54.7	-39.7
28	ER1-330	TATCGTATTTGCCCATCTCG	50.4	-38.1
29	ER1-423	CGGCAGCATAAGAGAAGGTC	51.6	-38.5
30	ER1-439	CCTTCCAGCTGCTTAACGGC	56.4	-41.9
31	ER1-450	CCAGATATTTGCCTTCCAGC	51.5	-38.8
32	ER1-479	ATAGATTTCGCCGGTCACGC	56.4	-41.8
33	ER1-495	GGAACTGGGCGCTCTCATAG	53.9	-39.7
34	ER1-504	GAATATAAAGGAACTGGGCG	48.5	-38.0
35	ER1-518	GCACGCGGCAACTAGAATAT	52.2	-39.4
36	ER1-529	TTCGAGAACAAGCACGCGGC	60.8	-43.3
37	ER1-543	TTTCACGCGGGTAGTTCGAG	55.2	-40.5
38	ER1-566	ACGCTTCACATATTGCAGGC	52.2	-38.7
39	ER1-584	GGAAACCGCGTCGTAAAAAC	53.9	-40.8
40	ER1-592	TTAAATGTGGAAACCGCGTC	52.7	-39.3
41	ER1-617	CATGATTGGCGTCGGCAGCG	64.0	-44.9
42	ER1-628	CGCACGCCGGACATGATTGG	63.8	-44.6
43	ER1-640	CGAGTCGGGGTACGCACGCC	64.2	-45.8
44	ER1-667	TCGATCAGTACGCAGGAGCT	52.4	-38.1
45	ER1-680	GCTGTCACCGCACTCGATCA	56.9	-39.1
46	ER1-689	GGAATCCAGGCTGTCACCGC	59.0	-41.9

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
47	ER1-704	GGAGGTGGCGTTGATGGAAT	56.0	-40.6
48	ER1-716	AACAATCGCGCTGGAGGTGG	59.5	-42.7
49	ER1-778	CTACCCAGCGCACGAATACG	55.7	-40.9
50	ER1-817	ATGCAGCCGGTATGGAACGC	59.4	-43.1
51	ER1-829	TTGTAGAACGGAATGCAGCC	52.8	-38.8
52	ER1-846	CCGCTGTCTGGAAATGTTTG	53.1	-38.6
53	ER1-855	AGGATTTCACCGCTGTCTGG	54.0	-39.2
54	ER1-874	CGCACACCGCCCTGAGAGCA	63.9	-44.0
55	ER1-907	CACATCGGGTAGAACAGCGT	52.5	-38.1
56	ER1-925	CTTTCCACTTCCAGATGCCA	52.5	-38.1
57	ER1-964	TTGCCTTCCACACCACGGTT	57.5	-40.8
58	ER1-971	CACGCGGTTGCCTTCCACAC	60.8	-42.5
59	ER1-981	CCATATGACGCACGCGGTTG	59.4	-42.1
60	ER1-1034	TTCACCTTTCAGCAGACGGG	55.0	-39.6
61	ER1-1055	CGGGCTGAACAGGGTGATAT	53.8	-39.6
62	ER1-1059	CGGACGGGCTGAACAGGGTG	62.1	-43.7
63	ER1-1061	GTCGGACGGGCTGAACAGGG	61.2	-43.4
64	ER1-1106	AAACTCTTCCTGATCGGCGA	53.8	-39.7
65	ER1-1148	GCGGATGCTGTCGTCTTTCT	54.3	-39.4
66	ER1-1155	GCTGCTTGCGGATGCTGTCG	61.3	-43.0
67	ER1-1166	GGCTTTCACACGCTGCTTGC	58.2	-41.4

SEQ ID No:	Name	Sequence 5'→3'	Tm (°C)	ΔG (kcal/mol)
68	ER1-1173	GCTCAACGGCTTTCACACGC	58.0	-41.3
69	ER1-1212	GACCGGTAGACGCACGTTCC	56.7	-40.8
70	ER1-1255	GGGCTATGGGTATTGCAGTG	52.1	-38.7
71	ER1-1259	AAACGGGCTATGGGTATTGC	53.3	-40.7
72	ER1-1265	CGGATCAAACGGGCTATGGG	58.7	-43.4
73	ER1-1311	GGGCTATCTCCAGGCACAGG	55.9	-40.7
74	ER1-1315	GGCAGGGCTATCTCCAGGCA	58.7	-42.5
75	ER1-1320	TGGTCGGCAGGGCTATCTCC	58.6	-42.4
76	ER1-1326	GCGGTTTGGTCGGCAGGGCT	64.9	-47.0
77	ER1-1330	TTCAGCGGTTTGGTCGGCAG	60.5	-43.1
78	ER1-1336	ACGTCGTTCAGCGGTTTGGT	56.8	-40.9
79	ER1-1356	TTTCACCGTTCTCGTCGTTG	53.5	-38.5
80	ER1-1364	CAGCGCGATTTCACCGTTCT	57.5	-41.7
81	ER1-1370	CGTACACAGCGCGATTTCAC	54.2	-38.9
82	ER1-1379	AGCAGACAGCGTACACAGCG	54.0	-38.2
83	ER1-1388	CAGGTTGAAAGCAGACAGCG	53.4	-38.4
84	ER1-1397	AATTGCGCCCAGGTTGAAAG	56.5	-41.9
85	ER1-1407	CCAGGTTATTAATTGCGCCC	53.8	-41.3
86	ER1-1428	TTGCCAGCTCTTCCAGTTCA	53.3	-38.2
87	ER1-1438	ACCGCCAGAATTGCCAGCTC	58.8	-42.5
88	ER1-1451	GTCAAGTGCACGAACCGCCA	59.1	-41.0

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
89	ER1-1463	ATCCAGCAGCGCGTCAAGTG	58.5	-41.2
90	ER1-1468	TGATAATCCAGCAGCGCGTC	56.1	-40.4
91	ER1-1535	GATCACACCAATACCCAGCG	52.6	-38.1
92	ER1-1561	TCGTTCGCCAGGTAGTAAGC	52.2	-39.0
93	ER1-1570	CGTTTACCGTCGTTCGCCAG	57.9	-42.2
94	ER1-1584	TGCCGTCGGAGTAGCGTTTA	55.8	-41.0
95	ER1-1605	TATGCGTCAGGTTGTTGGCG	56.8	-40.5
96	ER1-1614	CGAAGGTTTTATGCGTCAGG	52.5	-39.3
97	ER1-1688	GTTAAACCACGGGCACGCGC	62.0	-45.0
98	ER1-1705	TTCGCGTAAGTGGTTTCGTT	52.6	-39.3
99	ER1-1731	TATAGGTATCGATCGGCAGG	49.5	-38.0
100	ER1-1777	CAGTCGTAATGCAGCGGCTC	55.8	-40.2
101	ER1-1789	CGCAGAGCTTCCCAGTCGTA	55.4	-40.0
102	ER1-1839	TCAGAGCAGAAAGCGTGGAG	53.0	-38.1
103	ER1-1849	TCGGACGGCATCAGAGCAGA	58.9	-40.9
104	ER1-1874	GGCGTTAGAGATCTGCGAAG	51.8	-38.7
105	ER1-1916	TTTGATGCTGACGTAACCGC	53.7	-39.0
106	ER1-1923	TCGACGCTTTGATGCTGACG	57.1	-40.2
107	ER1-1944	CCTGGCGCAAAATACCGTCT	56.5	-42.0
108	ER1-1957	TAGTCCGGCACCACCTGGCG	62.5	-44.2
109	ER1-1968	GCAGGTGCTCGTAGTCCGGC	59.3	-42.4

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
110	ER1-1974	CGTCGTGCAGGTGCTCGTAG	56.7	-39.9
111	ER1-1983	GCTCATAGGCGTCGTGCAGG	58.0	-41.4
112	ER1-1992	CCCACAGCAGCTCATAGGCG	58.0	-41.5
113	ER1-2000	CGGCATTTCCCACAGCAGCT	59.7	-42.8
114	ER1-2010	CATCGTTACCCGGCATTTCC	56.5	-41.9
115	ER1-2083	GGATCGTAGTTGGTGTTGGC	51.8	-39.9
116	ER1-2112	TCGGCACTTTTCCTGACGGG	59.5	-42.8
117	ER1-2145	AGGCGGTGAGCAGGTCTTTC	55.7	-40.5
118	ER1-2154	CGAATTTGTAGGCGGTGAGC	54.8	-40.5
119	ER1-2166	GTGTTTTGACCCCGAATTTG	51.9	-38.6
120	ER1-2211	CGTCTTGTGCGTCTTCAGCG	56.8	-40.0
121	ER1-2262	TCTTACATGCGCCGCTTTCG	58.6	-42.8

Table 2

Antisense oligonucleotides that target the *Escherichia coli* K12 ribonucleotide reductase small subunit (R2)

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
122	ER2-50	CGGCTGACCAAAGAACATCG	55.5	-40.0
123	ER2-60	CCACGTTGACCGGCTGACCA	61.2	-42.2
124	ER2-67	TAGCGAGCCACGTTGACCGG	60.6	-43.2
125	ER2-134	CGGACGCCAGAAGAAAGAGA	54.4	-39.8
126	ER2-144	CAACTTCTTCCGGACGCCAG	57.0	-41.3

SEQ ID No:	Name	Sequence 5'→3'	Tm (°C)	ΔG (kcal/mol)
127	ER2-168	AATCTATACGGTCGCGGGAG	53.4	-40.5
128	ER2-198	TGTGTTTTTCGTGCTCCGGC	58.3	-41.6
129	ER2-273	GCAATAGCGCCACGTTCGGG	62.1	-45.2
130	ER2-284	AGAAATAAGCGGCAATAGCG	51.8	-40.3
131	ER2-290	CGGAATAGAAATAAGCGGCA	52.4	-40.3
132	ER2-307	ACCCAGGTTTCCAGTTCCGG	57.4	-42.0
133	ER2-350	ATAGGAACGGGAATGAATCG	50.7	-38.8
134	ER2-441	TCCCTTCCGCACGTTTCTGG	59.5	-42.8
135	ER2-498	CGCCCAGCAGATGCCAGTAG	58.0	-41.5
136	ER2-505	GTACCTTCGCCCAGCAGATG	54.6	-39.7
137	ER2-544	CGCAGGCTAACGGTCACAGT	55.2	-39.7
138	ER2-557	TTTCTTCAGCTCGCGCAGGC	60.2	-43.4
139	ER2-640	GCAAATGCGAAGGAACAAGC	54.9	-40.4
140	ER2-655	ATCAATTCGCGTTCTGCAAA	53.4	-39.3
141	ER2-680	GCGAATAATTTTGGCGTTGC	54.9	-41.6
142	ER2-692	GCGGGCAATCAGGCGAATAA	59.5	-44.0
143	ER2-704	CAGGGCTTCGTCGCGGGCAA	66.8	-47.8
144	ER2-714	CGGTCAGGTGCAGGGCTTCG	62.3	-44.0
145	ER2-724	TGCTGGGTGCCGGTCAGGTG	63.6	-43.5
146	ER2-728	CATATGCTGGGTGCCGGTCA	58.8	-41.4
147	ER2-778	GCAATTTCCGCCATCTCAGG	56.8	-41.5

SEQ ID No:	Name	Sequence 5'→3'	Tm (°C)	ΔG (kcal/mol)
148	ER2-796	TCCTGCTTACACTCTTCGGC	52.1	-38.3
149	ER2-848	ATCCGCCCAGTCTTTCTCCT	54.2	-40.4
150	ER2-857	GAACAGATAATCCGCCCAGT	50.7	-38.1
151	ER2-976	GGGTTGGAGCGCGTCTGGAA	61.8	-44.0
152	ER2-983	CGGGATCGGGTTGGAGCGCG	68.1	-49.1
153	ER2-985	CACGGGATCGGGTTGGAGCG	64.0	-45.6
154	ER2-1045	CTGACTTCCACTTCCTGCGG	54.6	-39.9
155	ER2-1063	TGCCCGACCAGATAAGAACT	51.3	-38.2
156	ER2-1076	TTCCGAGTCAATCTGCCCGA	57.8	-41.2
157	ER2-1092	AATCGTCGGTGTCCACTTCC	53.6	-38.8

Table 3
Antisense Sequences that Target *Escherichia coli SecA*

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/mol
158	ES56	GACCACTTTGCGCATCCGGC	62.1	-44.2
159	ES62	GATGTTGACCACTTTGCGCA	54.3	-38.3
160	ES85	ATCTCCGGTTCCATGGCATT	55.5	-40.8
161	ES92	TTTTTCCATCTCCGGTTCCA	54.3	-40.1
162	ES116	CCCTTTCAGTTCTTCGTCGG	53.8	-39.8
163	ES124	GCGGTTTTCCCTTTCAGTTC	52.9	-39.9
164	ES129	ACTCTGCGGTTTTCCCTTTC	52.5	-39.6
165	ES153	CGCCTTTTTCCAGACGTGCA	58.4	-41.9
166	ES158	CACTTCGCCTTTTTCCAGAC	51.5	-38.4
167	ES165	TTTCCAGCACTTCGCCTTTT	54.1	-40.5

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/mol
168	ES170	CAGATTTTCCAGCACTTCGC	52.5	-38.6
169	ES206	ACTTGCCTCACGTACCACGG	54.9	-39.5
170	ES215	GACGCGCTTACTTGCCTCAC	55.0	-40.1
171	ES230	GTGACGCATACCAAAGACGC	53.1	-38.5
172	ES264	TAAGAACCATACCGCCGAGT	51.5	-39.1
173	ES286	ATTTCGGCGATGCAGCGTTC	59.7	-43.4
174	ES303	TTCCTTCACCGGTACGCATT	54.5	-40.3
175	ES307	GTTTTTCCTTCACCGGTACG	51.4	-38.9
176	ES320	CGTTGCGGTCAGGGTTTTTC	56.8	-41.6
177	ES336	TCAGGTAAGCAGGCAGCGTT	55.0	-40.2
178	ES351	TACCGGTTAGTGCGTTCAGG	52.8	-39.2
179	ES392	TTGCGCCAGGTAGTCGTTGA	56.5	-40.4
180	ES398	GTCACGTTGCGCCAGGTAGT	55.0	-39.5
181	ES418	AGCGGACGGTTGTTTTCGGC	60.8	-44.5
182	ES429	GGAATTCAAACAGCGGACGG	56.7	-41.5
183	ES436	AGGCCAAGGAATTCAAACAG	51.0	-38.4
184	ES448	ATACCGACAGTCAGGCCAAG	51.6	-38.0
185	ES485	TTCGCGCTTTGCCGGTGCTG	65.8	-46.9
186	ES531	AGCCGTATTCGTTGTTCGTA	50.1	-37.9
187	ES544	CGCAGGTAGTCAAAGCCGTA	53.1	-39.5
188	ES553	ATGTTGTCGCGCAGGTAGTC	52.6	-38.1
189	ES556	GCCATGTTGTCGCGCAGGTA	59.2	-41.7
190	ES617	GTCCACTTCGTCCACCAGCG	57.7	-40.4
191	ES646	GGTGTACGCGCTTCATCGAT	55.0	-40.0
192	ES647	CGGTGTACGCGCTTCATCGA	59.3	-42.1
193	ES695	GCGTTTATACATTTCCGAGC	49.5	-38.4
194	ES724	CGGATCAGGTGCGGAATAAT	53.9	-40.4

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/mol
195	ES799	TTCACCTGGCGAGATTTTTC	51.8	-38.6
196	ES824	CAGCACCAGACCACGTTCGG	58.6	-40.7
197	ES851	GCCCTCTTTCACCAGCAGTT	53.3	-39.1
198	ES866	CCCTTCATCCATGATGCCCT	55.9	-40.6
199	ES889	TTGGCCGGAGAGTACAGAGA	52.2	-38.1
200	ES898	AGCATGATGTTGGCCGGAGA	57.6	-40.9
201	ES922	AGCGCCGCCGTTACGTGGTG	64.6	-46.5
202	ES950	GTCACGGGTAAACAGCGCAT	54.9	-40.0
203	ES1068	CACCTTCTTTCGCTTCCACA	52.8	-38.4
204	ES1097	CAGCGTTTGGTTTTCGTTCT	52.1	-38.9
205	ES1109	GGTGATCGAAGCCAGCGTTT	56.5	-41.2
206	ES1128	GACGGAAGTAGTTCTGGAAG	45.5	-35.0
207	ES1147	CCCGCCAGTTTTTCATACAG	52.3	-39.2
208	ES1152	TCATCCCCGCCAGTTTTTCA	57.5	-41.6
209	ES1218	GAACAACGACGGTATCCAGC	52.0	-38.2
210	ES1328	GCCTTTCGCAGTACGTTCTT	51.4	-38.9
211	ES1350	TAGTACCCACCAGCACCGGC	57.1	-41.4
212	ES1398	CGGCTTTGGTCAGTTCGTTT	54.3	-40.1
213	ES1410	TGTGCTTAATACCGGCTTTG	50.8	-38.6
214	ES1439	GTTGGCGTGGAATTTGGCGT	59.3	-43.0
215	ES1462	GCCTGAGCAACAATCGCCGC	62.4	-44.5
216	ES1515	CTGTACCACGACCCGCCATA	55.6	-40.3
217	ES1518	TATCTGTACCACGACCCGCC	54.7	-40.0
218	ES1545	CTGCCTGCCAGCTACCACCG	60.2	-42.9
219	ES1563	TTTCCAGCGCGGCAACTTCT	59.4	-43.4
220	ES1581	TTTGCTCTGCGGTCGGATTT	57.0	-41.8
221	ES1589	TTTTTCAATTTGCTCTGCGG	53.2	-39.8

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/mol
222	ES1624	ACCGCATCGTGACGTACCTG	55.7	-39.6
223	ES1629	CCAGTACCGCATCGTGACGT	55.7	-39.6
224	ES1633	GCTTCCAGTACCGCATCGTG	55.5	-40.0
225	ES1655	ACCGATGATATGCAGGCCAC	54.6	-39.6
226	ES1712	ACGACCAGAACGACCGCGCA	63.3	-44.1
227	ES1718	CCCCTGACGACCAGAACGAC	56.6	-40.1
228	ES1722	CATCCCCCTGACGACCAGAA	56.9	-40.4
229	ES1739	GAAACGGGAAGAACCAGCAT	53.1	-39.5
230	ES1748	CGACAGGTAGAAACGGGAAG	51.4	-38.6
231	ES1781	GGAAGCAAAAATACGCATCA	50.6	-38.2
232	ES1785	GGTCGGAAGCAAAAATACGC	53.9	-40.9
233	ES1794	CGGATACTCGGTCGGAAGCA	57.3	-41.7
234	ES1814	ACCCAGTTTACGCATCATGC	52.5	-38.5
235	ES1845	ACGGGTGTTCAATGGCTTCG	57.1	-41.2
236	ES1861	ATCGCTTTAGTCACCCACGG	54.1	-40.0
237	ES1888	CTTTCAACTTTACGCTGGGC	51.9	-39.3
238	ES1892	ACGGCTTTCAACTTTACGCT	51.1	-39.2
239	ES2007	TGGTTTCGCTCACATCGCTG	57.0	-40.0
240	ES2054	GTAGGCATCAATGGTCGCTT	51.7	-38.5
241	ES2084	CCACATTTCTTCCAGCGACT	51.7	-38.0
242	ES2087	ATCCCACATTTCTTCCAGCG	53.9	-39.7
243	ES2191	TCACGCAGCGTCTCTTCATG	54.7	-38.2
244	ES2275	CCTTTCTCGAAGTGACGCAT	51.9	-38.2
245	ES2306	CCACAGGGAGTCAAGCGTTT	54.1	-39.3
246	ES2325	TCGCTGCCAGGTGCTCTTTC	57.7	-41.1
247	ES2330	GTCCATCGCTGCCAGGTGCT	59.7	-41.9
248	ES2339	ACGCAGATAGTCCATCGCTG	52.7	-38.4

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/mol
249	ES2381	CTTCGGATCTTTCTGTGCGT	51.9	-38.2
250	ES2395	CGTTTGTATTCCTGCTTCGG	52.5	-39.4
251	ES2422	ATCGCTGCAAACATGGAGAA	53.1	-38.5
252	ES2520	CCATACGACGCTGTTGTTCC	52.9	-38.5
253	ES2525	GGCTTCCATACGACGCTGTT	54.2	-40.0
254	ES2537	CGCTAAACGCTCGGCTTCCA	59.9	-44.1
255	ES2555	GCTAAGCTGCTGCATTTGCG	56.2	-41.3
256	ES2619	CTACTTTGCGCTCTCCGGTT	53.8	-40.4
257	ES2626	TTACGTCCTACTTTGCGCTC	50.0	-38.0
258	ES2646	AACCGCACGGGCAAGGATCG	63.6	-45.9
259	ES2651	ACCAGAACCGCACGGGCAAG	61.7	-44.0
260	ES2656	TTTTTACCAGAACCGCACGG	55.1	-41.0

Table 4
Antisense Sequences that Target *E. coli SecA* based on Conserved Sequences

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/mol
261	ES386	CAGGTAGTCGTTGACGGTAA	47.7	-35.7
262	ES388	CAGGTAGTCGTTGACGGT	45.0	-32.9
263	ES1126	CGGAAGTAGTTCTGGAAGGT	47.6	-36.5
264	ES1702	CGACCGCGCAACTGGTTATC	57.8	-41.9
265	ES2644	CCGCACGGGCAAGGATCGTT	63.6	-45.9

In Tables 1, 2, 3, and 4, the "Tm" is the melting temperature of an oligonucleotide duplex calculated according to the nearest-neighbor thermodynamic values. At this temperature 50% of nucleic acid molecules are in duplex and 50% are denatured. The "ΔG" is the free energy of the oligonucleotide, which is a measurement of an oligonucleotide duplex stability.

The following sequences have been determined to be conserved among species:

ES386 [SEQ ID NO:261] is conserved among *Escherichia coli* and *Mycobacterium tuberculosis*;

ES388 [SEQ ID NO:262] is conserved among *Escherichia coli; Mycobacterium tuberculosis*; and *Mycobacterium bovis*;

ES553 [SEQ ID NO:188] is conserved among *Escherichia coli*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Streptomyces coelicolor*; and *Streptomyces lividans*;

ES556 [SEQ ID NO:189] is conserved among *Escherichia coli*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Streptomyces coelicolor*; and *Streptomyces lividans*; and *Synechoccus sp.*; and

ES646 [SEQ ID NO:191] is conserved among *Escherichia coli* and *Staphylococcus carnosus*;

ES1126 [SEQ ID NO:263] is conserved among *Escherichia coli* and *Rhodobacter capsulatus* SecA genes.

ES2644 [SEQ ID NO:265] is conserved among *Escherichia coli* SecA gene, MutA (A:T to C:G transversion), and tyrosine-specific transport protein (tyrP) gene.

The term "alkyl" refers to monovalent alkyl groups preferably having from 1 to 20 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, and the like.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is either fluoro or chloro.

The term "thiol" refers to the group -SH.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the antisense oligonucleotides of this invention and which are not biologically or otherwise undesirable. In many cases, the antisense oligonucleotides of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl,

heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "ribonucleotide reductase gene" or the "ribonucleoside diphosphate reductase gene" refers to any gene which encodes a protein that either reduces the four main ribonucleotides to the corresponding deoxyribonucleotides involved in DNA synthesis or encodes a subunit of a multimeric enzyme which reduces the four main ribonucleotides to the corresponding deoxyribonucleotides. Without being limiting, examples of ribonucleotide reductase genes from bacteria include the *E. coli* nrdA, nrdB and nrd D genes; the *S. typhimurium* nrdE and nrdF genes; and the *Lactococcus lactis* nrdEF gene. Examples of the ribonucleotide reductase genes from viruses include the herpes simplex type 1 and 2 ribonucleotide reductases and the bovine and equine herpes simplex ribonucleotide reductases.

The term "secA" refers to an oligonucleotide sequence which encodes a protein having similar properties as those expressed by the *E. coli* secA gene. Without being

limiting, examples of secA genes from bacteria include the *Mycobacterium bovis* secA gene; the *Mycobacterium tuberculosis* secA gene, the *Staphylococcus aureus* secA gene and the *Staphylococcus carnosus* secA gene.

The term "microorganism" means a bacteria, fungi or virus having either a ribonucleotide reductase or secA gene. Specifically excluded from this definition is the malerial parasite, plasmodium.

The term "bacteria" refers to any bacteria encoding either a ribonucleotide reductase gene or a secA gene, including *Escherichi coli*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium smegmatis*, *Salmonella typhimurium*, *Thermoplasma acidophilum*, *Pyrococcus furiosus*, *Bacillus subtilis*, *Bacillus firmus*, *Lactococcus lactis*, *Staphylococcus aureus*, *Staphylococcus carnosus*, *Listeria monocytogenes*, *Borrelia burgdorferi*, *P. sativum*, *S. griseus*, and *Synechoccus sp.*

The term "virus" refers to any virus having a ribonucleotide reductase gene. Preferably the virus will be a DNA virus. Examples of suitable viruses include various herpes viruses (such as herpes simplex types 1 and 2, varicella-herpes zoster, cytomegalovirus and Epstein-Barr virus) and the various hepatitis viruses.

The term "complementary to" means that the antisense oligonucleotide sequence is capable of binding to the target sequence, ie the ribonucleotide reductase gene or the secA gene. Preferably the antisense oligonucleotide sequence has at least about 75% identity with the target sequence, preferably at least about 90% identity and most preferably at least about 95% identity with the target sequence allowing for gaps or mismatches of several bases. Identitiy can be determined, for example, by using the BLASTN program of the University of Wisconsin Computer Group (GCG) software.

The term "inhibiting growth" means a reduction in the growth of the bacteria or viruses of at least 25%, more preferably of at least 50% and most preferably of at least 75%. The reduction in growth can be determined for bacteria by a measuring the optical density of a liquid bacteria culture with a spectrophotometer or by counting the number of colony forming units/ml (CFU/ml) upon plating on culture plates. The reduction in growth can be determined for viruses by measuring the number of plaque forming units/ml upon plating on susceptible cells.

Preparation of the Antisense Oligonucleotides

The antisense oligonucleotides of the present invention may be prepared by conventional and well-known techniques. For example, the oligonucleotides may be prepared using solid-phase synthesis and in particular using commercially available equipment such as the equipment available from Applied Biosystems Canada Inc., Mississauga, Canada. The oligonucleotides may also be prepared by enzymatic digestion of the naturally occurring ribonucleotide reductase or secA gene by methods known in the art.

Isolation and Purification of the Antisense Oligonucleotides

Isolation and purification of the antisense oligonucleotides described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. However, other equivalent separation or isolation procedures could, of course, also be used.

The invention contemplates a method of evaluating if an antisense oligonucleotide inhibits the growth of a microbe having a ribonucleotide reductase or secA gene. The method comprises selecting the microbe/microorganism having a ribonucleotide reductase or secA gene, administering the antisense oligonucleotide; and comparing the growth of the treated microbe with the growth of an untreated microorganism.

In order for the antisense oligonucleotide to effectively interrupt the expression of the ribonucleotide reductase or secA gene, the antisense oligonucleotide enters the microorganism's cell, in the case of fungal or bacterial cells or enter the mammalian cell having the virus target.

Although oligonucleotides are taken up by bacterial cells, some modification of the oligonucleotides may help facilitate or regulate said uptake. thus, a carier molecule, for example an amino acid, can be linked to the oligonucleotide. for example, bacteria have multiple transport systems for the recognition and uptake of

molecules of leucine. The addition of this amino acid to the oligonucleotide may facilitate the uptake of the oligonucleotide in the bacteria and not substantially interfere with the activity of the antisense oligonucleotide in the bacterial cell.

Other methods are contemplated for facilitating the uptake of the antisense oligonucleotide into bacteria. For example, the addition of other amino acids or peptides or primary amines to the 3' or 5' termini of the antisense oligonucleotide may enable utilization of specific transport systems. Addition of lactose to the oligonucleotide by a covalent linkage may also be used to enable transport of the antisense oligonucleotide by lactose permease. Other sugar transport systems are also known to be functional in bacteria and can be utilized in this invention.

With regard to inhibiting the expression of ribonucleotide reductase in DNA viruses, the antisense oligonucleotide is preferably introduced into the cell infected with the DNA virus. The antisense oligonucleotides may be delivered using vectors or liposomes.

An expression vector comprising the antisense oligonucleotide sequence may be constructed having regard to the sequence of the oligonucleotide and using procedures known in the art. The vectors may be selected from plasmids or benign viral vectors depending on the eukaryotic cell and the DNA virus. Phagemids are a specific example of beneficial vectors because they can be used either as plasmids or a bacteriophage vectors. Examples of other vectors include viruses such as bacteriiophages, baculoviruses and retroviruses, DNA viruses, liposomes and other recombination vectors.

Vectors can be constructed by those skilled in the art to contain all the expression elements required to achieve the desired transcription of the antisense oligonucleotide sequences. Therefore, the invention provides vectors comprising a transcription control sequence operatively linked to a sequence which encodes an antisense oligonucleotide. Suitable transcription and translation elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes. Selection of appropriate elements is dependent on the host cell chosen.

Reporter genes may be included in the vector. Suitable reporter genes include β-galactosidase (e.g. lacZ), chloramphenicol, acetyl-transferase, firefly luciferase, or an immunoglobulin or portion thereof. Transcription of the antisense oligonucleotide may be monitored by monitoring for the expression of the reporter gene.

The vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al.[18]; Ausubel et al.[19]; Chang et al.[20]; Vega et al.[21]; and Vectors: A Survey of Molecular Cloning Vectors and Their Uses[22] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.

Introduction of nucleic acids by infection offers several advantages. Higher efficiency and specificity for tissue type can be obtained. Viruses typically infect and propagate in specific cell types. Thus, the virus' specificity may be used to target the vector to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

Pharmaceutical Formulations

When employed as pharmaceuticals, the antisense oligonucleotides are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the antisense oligonucleotides associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or

liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the antisense oligonucleotide is employed at no more than about 20 weight percent of

the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The antisense oligonucleotide is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the antisense oligonucleotide actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient/antisense oligonucleotide is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with

edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

Formulation Example 9

A formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	5.0 mg
Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the antisense oligonucleotides of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, for example, U.S. Patent 5,023,252[23], herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Another preferred method of delivery involves "shotgun" delivery of the naked antisense oligonucleotides across the dermal layer. The delivery of "naked" antisense oligonucleotides is well known in the art. See, for example, Felgner et al.,U.S. Patent No. 5,580,859[24]. It is contemplated that the antisense oligonucleotides may be packaged in a lipid vesicle before "shotgun" delivery of the antisense oligonucleotide.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472[25] which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences*[26].

The antisense oligonucleotides or the pharmaceutical composition comprising the antisense oligonucleotides may be packaged into convenient kits providing the necessary materials packaged into suitable containers.

Utility

The antisense oligonucleotides of the present invention may be used for a variety of purposes. They may be used to inhibit the expression of the ribonucleotide reductase gene in a microorganism, resulting in the inhibition of growth of that microorganism. They may be used to inhibit the expression of the secA gene in a microorganism, resulting in the inhibition of growth of that microorganism. The oligonucleotides may be used as hybridization probes to detect the presence of the ribonucleotide reductase gene or the secA gene in the microorganism. When so used the oligonucleotides may be labeled with a suitable detectable group (a radioisotope, a ligand, another member of a specific binding pair, for example, biotin). The oligonucleotides may also be used to determine the presence of a particular

microorganism in a biological sample. Finally, the oligonucleotides may be used as molecular wight markers.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given but are not meant to limit the scope of the claims in any way.

EXAMPLES

In the examples below, all temperatures are in degrees Celsius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated).

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning:

μM	=	micromolar
mM	=	millimolar
M	=	molar
ml	=	milliliter
μl	=	microliter
mg	=	milligram
μg	=	microgram
IPTG	=	isopropyl-β-D-thiogalactoside
PAGE	=	polyacrylamide gel electrophoresis
PVDF	=	polyvinylidene difluoride
rpm	=	revolutions per minute
OD	=	optical density
CFU	=	colony forming units
ΔG	=	free energy, a measurement of oligonucleotide duplex stability
kcal	=	kilocalories

General Methods in Molecular Biology:

Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al.[18]; Ausubel et al.[19]; and Perbal[27].

The antisense oligonucleotides in Tables 1, 2 and 3 were selected from the sequence complementary to the ribonucletide reductase or secA genes of *E. coli* such that the sequence exhibited the least likelihood of showing one or more of duplex formation, hair-pin formation, and homooligomer/sequence repeats but had a high to moderate potential to bind to the ribonucleotide reductase gene or the secA gene sequence. These properties were determined using the computer modeling program OLIGO Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN).

The antisense oligonucleotides in Table 4 were selected on the basis that the sequence is highly conserved for the secA genes between two or more microbial species. This property was determined using the BLASTN program (Altschul, et al.[16]) of the University of Wisconsin Computer group (GCG) software (Devereux J. et al.[17]) with the National Center for Biotechnology Information (NCBI) databases

Phosphorothioate oligonucleotides comprising the desired sequences were specially ordered either from Boston BioSystems, Bedford MA; Canadian Life Technologies, Burlington, Canada; Dalton Chemical Laboratories, Inc., North York, Canada; Hybridon, Inc., Milford Ma; Oligos Etc., or Oligos Therapeutics, Inc., Wilsonvill OR; or TriLink Bio Technologies, San Diego, CA. Antisense oligonucleotides may also be made by methods known in the art.

Polymerase chain reaction (PCR) was carried out generally as in *PCR Protocols: A Guide To Methods And Applications*[28].

Example 1: Inhibition of mouse ribonucleotide reductase small subunit (R2) expression in *Escherichia coli by antisense oligonucleotide AS-II-626-20*

Competent BL21 (DE3) cells carrying a plasmid containing the mouse ribonucleotide reductase R2 gene were used. (Mann et al.[34]) The antisense oligonucleotide, AS-II-626-20, GGCTAAATCGCTCCACCAAG [SEQ ID NO:266] is specifically complementary to the mouse ribonucleotide reductase R2 gene. Approximately 10^{10} bacteria/ml were electroporated using a Cell Porator (Gibco BRL, Burlington, Canada) in micro electro-chambers (0.4 cm between the electrodes) at a pulse of 2.4 kV, 4 kΩ with either 20 μM or 200 μM of antisense oligonucleotide AS-II-626-20, following methods described by the manufacturer (Dower W.J.[29] ; Neuman et; and Taketo, A.[31]). Control populations were subjected to electroporation but without the antisense oligonucleotide AS-II-626-20.

The bacterial cells were then transferred to Luria-Bertani broth (Miller J.H.[32]) containing 50 μg/ml of ampicillin and 0.4 mM of isopropyl β-D-thiogalactoside (IPTG) (expression inducer) (Horwitz J.P.[33]) to grow at 30°C on a shaker at 250 rotations per minute (rpm) for 5 hours.

The cells were harvested by centrifugation and treated with 2 x sample loading buffer (100 mM Tris[hydroxymethyl'aminomethane, pH 6.8, 200 mM dithiothrietol, 4% sodium dodecyl sulfate, 20% glycerol and 0.015% bromophenol blue) and sonicated (Olsvik, et al.[35]) for 15 seconds. The supernatants were resolved by polyacrylamide gel electrophoresis (PAGE) (Laemmli U.K.[36]).

The ribonucleotide reductase R2 expression was detected by Western blot. The protein gel was blotted onto polyvinylidene difuoride (PVDF) protein sequencing membrane. (Choy et al.[37]). The presence of the mouse ribonucleotide reductase was detected with a rabbit anti-mouse R2 subunit antibody (Chan et al.[39]). The presence of the antibody bound to the ribonucleotide reducatase was detected using a second goat anti-rabbit immunoglobulin linked with horseradish peroxidase (Amersham Life Sciences, Oakville Canada).

The upper panel of Figure 14 is a photograph of the Western Blot results. The lower panel of Figure 14 is a photograph of the membrane stained with India ink to indicate the level of protein loaded in each lane.

It is clear that administration of either 20 μM or 200 μM AS-II-626-20 resulted in a marked reduction of mouse ribonucleotide reductase gene expression in the *E. coli* cells.

Example 2: Inhibition of bacteria *Escherichia coli* K12 growth by antisense oligonucleotides ER1-169 and ER2-724 targeting *E. coli* ribonucleotide reductase large subunit (R1) and small subunit (R2)

E. coli cells were electroporated by the method set forth in Example 1 with ER1-169 [SEQ ID NO:22] or ER2-724 [SEQ ID NO:145] at the concentrations shown in Figure 15, while the control cells received oligonucleotide AS-II-626-20 [SEQ ID NO:] (targeting mouse ribonucleotide reductase small subunit).

The *E. coli* cells were then transferred to fresh Luria-Bertani broth (Miller J.H.[32]) to grow at 30°C on a shaker at 250 rpm for 3 hours. The flasks for the test and the control each contained the same number of bacteria per ml at the start of the experiment. The optical density at 590 nm (OD_{590}) of the cultures was measured at the start and at the end of the 3 hours. The inhibition of *E. coli* growth was calculated by comparing the increase in OD_{590} values at the start and the end of the 3 hours of the oligonucleotide-treated cultures to the increase of the control cultures at the start and at the end of the 3 hours. (Carpentier P.L.[40])

The results indicate that ER1-169 [SEQ ID NO:22] and ER2-724 [SEQ ID NO:145] inhibited the growth of *E. coli*.

Example 3: Killing of *Escherichia coli* K12 by antisense oligonucleotides targeting the ribonucleotide reductase large subunit (R1) or the small subunit (R2)

E. coli cells (approximately 2 x 10^9 were incubated with 20 μM of each of the phosphorothioate oligonucleotides set forth in Figure 12 on ice for 45 minutes. A

control without oligonucleotides was also incubated for each experiment. Cells were heat shocked by placing them in a 42°C bath for 45 seconds. (Sambrook J. et al.[18])

Luria-Bertani (LB) broth (Miller J.H.[32]) was added and the samples were incubated at room temperature for 30 minutes. Dilutions of treated and untreated bacteria were incubated overnight at 37°C on culture plates containing LB medium, and the number of colonies was counted.

The number of killed bacteria was calculated by subtracting the surviving colony forming units (CFU/ml) of the oligonucleotide-treated bacteria from the CFU/ml of the control. Figure 16 shows the number of bacteria killed by treatment with the antisense sequences: ER1-640 [SEQ ID NO:43]; ER1-1059 [SEQ ID NO:62]; ER1-1320 [SEQ ID NO:75]; ER1-1315 [SEQ ID NO:74]; ER1-1326 [SEQ ID NO:76]; ER2-704 [SEQ ID NO:143] and ER2-983 [SEQ ID NO:152].

The results from Figure 16 show that antisense oligonucleotides complementary to either the R1 or R2 subunit of ribonucleotide reductase are effective as anti-bacterial agents.

Example 4: Inhibition of the secA protein expression in Escherichia coli following treatment with antisense phosphorothioate oligonucleotides

E. coli cells were heat shock transformed by the method set forth in Example 3 above with the 80 μM of each of the antisense phosphorothioate oligonucleotides set forth in Figure 17.

Luria-Bertani broth was then added to the treated *E. coli* cells and they were allowed to grow at 30°C on a shaker at 250 rpm for 3 hours.

Approximately the same quantity of treated and untreated bacteria, based on optical density, were washed in phosphate buffered saline, suspended in 2X Laemmli sample buffer (Laemmli U.K.[36]), heated for 5 minutes at 95°C and subjected to SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis).

The gel was blotted onto polyvinylidene difluoride protein sequencing membrane by the methods set forth in Example 1. A rabbit polyclonal SecA antiserum (der Blaauwen et al.[6]) was used to detect the expression of the *E. coli* secA gene. The presence of bound rabbit antibody was detected using a goat anti-rabbit immunoglobulin (Amersham Life Sciences, Oakville, Canada).

Figure 17 is a photograph of the Western Blot of *E. coli* cells treated with oligonucleotides ES799 [SEQ ID NO:195] (lane 1); ES1845 [SEQ ID NO:235] (lane 2); and the control (lane 3). When compared to the control, lane 3, the ES799 [SEQ ID NO:195] and ES1845 [SEQ ID NO:235] oligonucleotides clearly decreased the SecA protein levels in the treated *E. coli* cells. The top band in the Figure 17 represents SecA. Non-specific background bands appear below the SecA protein band.

It has been found that the antisense oligonucleotides are effective inhibitors of SecA expression in *E. coli*.

Example 5: Killing of Escherichia coli K12 by antisense secA oligonucleotides

E. coli cells were heat shock transformed by the method described in Example 3 above with either 100 μM or 20 μM of the antisense phosphorothioate oligonucleotides set forth in Figures 18a and 18b

Luria-Bertani (LB) broth (Miller J.H.[32]) was added and the bacterial samples were incubated at room temperature for 30 minutes. Dilutions of treated and untreated bacteria were incubated overnight at 37°C on culture plates containing LB medium, and the number of colonies was counted.

The number of killed bacteria was calculated by subtracting the surviving colony forming units (CFU/ml) of the oligonucleotide-treated bacteria from the CFU/ml of the control. Figures 18a and 18b show the number of bacteria killed by treatment with the various antisense sequences. Accordingly, antisense oligonucleotides complementary to the secA gene act to inhibit the growth of *E. coli*.

Example 6: Effect of antisense oligonucleotides on Escherichia coli K12 growth

E. coli cells were heat shock transformed by the method described in Example 3 with either 16 μM, 20 μM or 80 μM of each of the antisense phosphorothioate oligonucleotides set forth in Figures 19a-g.

Equal numbers of the treated *E. coli* cells were then transferred to flasks containing fresh Luria-Bertani broth to grow at 30°C on a shaker at 250 rpm. The number of bacteria per flask was determined by the turbidity of the cultures at OD_{620} taken each hour (Carpentier P.L.[40]).

Figures 19a-g show the rate of growth of the *E. coli* in each of the flasks after treatment with the various oligonucleotides. When growth curves of the treated and untreated cultures were statistically analyzed, the growth of the antisense treated cultures was found to be significantly inhibited when compared to the control cultures. The statistical p values are found in the Figures.

Claims:

1. An antisense oligonucleotide which is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism.

2. The oligonucleotide of Claim 1 comprising one or more phosphorothioate internucleotide linkages.

3. An antisense oligonucleotide comprising from about 3 to about 50 nucleotides which is capable of binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

4. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an oligonucleotide which is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism.

5. The pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an oligonucleotide comprising from about 3 to about 50 nucleotides which is capable of binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143;

SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

6. A method of inhibiting the expression of a ribonucleotide reductase gene in a microorganism having a ribonucleotide reductase gene, comprising administering to said microorganism or to a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to the ribonucleotide reductase gene of the microorganism under conditions such that the expression of the ribonucleotide reductase gene is inhibited.

7. The method according to Claim 6, wherein said microorganism is a bacterial cell.

8. The method according to Claim 6, wherein said microorganism is a virus.

9. The method according to Claim 6 wherein the antisense oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; and SEQ ID NO:152.

10. A method of inhibiting the expression of the secA gene in a microorganism having a secA gene, comprising administering to said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to the secA gene of the microorganism under conditions such that the secA gene is inhibited.

11. The method according to Claim 10, wherein said microorganism is a bacterial cell.

12. The method according to Claim 11 wherein the antisense oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

13. A method of inhibiting the growth of a microorganism having a ribonucleotide reductase gene or a secA gene, which method comprises identifying the microorganism and administering to said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions whereby the growth of the microorganism is inhibited.

14. The method according to Claim 13, wherein said microorganism is a bacterial cell.

15. The method according to Claim 13, wherein said microorganism is a virus.

16. The method according to Claim 13 wherein the antisense oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

17. A method for treating a mammalian pathologic condition mediated by microorganisms, which method comprises identifying a mammal having a pathologic condition mediated by microorganisms having a ribonucleotide reductase gene or a secA gene and administering to said mammal an effective amount of an antisense oligonucleotide comprising at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited.

```
   1 atgaatcaga atctgctggt gacaaagcgc gacggtagca cagagcgcat caatctcgac
  61 aaaatccatc gcgttctgga ttgggcggca gaaggactgc ataacgtttc gatttcccag
 121 gtcgagctgc gctcccacat tcagttttat gacggtatca agacctctga catccacgaa
 181 accattatca aggctgccgc agacctgatc tcccgtgatg cgccggatta tcagtatctc
 241 gccgcgcgcc tggcgatctt ccacctgcgt aaaaaagcct acggccagtt tgagccgcct
 301 gcgctgtacg accacgtggt gaaaatggtc gagatgggca aatacgataa tcatctgctg
 361 gaagactaca cggaagaaga gttcaagcag atggacacct ttatcgatca cgaccgtgat
 421 atgaccttct cttatgctgc cgttaagcag ctggaaggca aatatctggt acagaaccgc
 481 gtgaccggcg aaatctatga gagcgcccag ttcctttata ttctagttgc cgcgtgcttg
 541 ttctcgaact acccgcgtga aacgcgcctg caatatgtga agcgttttta cgacgcggtt
 601 tccacattta aaatttcgct gccgacgcca atcatgtccg gcgtgcgtac cccgactcgt
 661 cagttcagct cctgcgtact gatcgagtgc ggtgacagcc tggattccat caacgccacc
 721 tccagcgcga ttgttaaata cgtttcccag cgtgccggga tcggcatcaa cgccgggcgt
 781 attcgtgcgc tgggtagccc gattcgcggt ggtgaagcgt tccataccgg ctgcattccg
 841 ttctacaaac atttccagac agcggtgaaa tcctgctctc agggcggtgt gcgcggcggt
 901 gcggcaacgc tgttctaccc gatgtggcat ctggaagtgg aaagcctgct ggtgttgaaa
 961 aacaaccgtg gtgtggaagg caaccgcgtg cgtcatatgg actacggggt acaaatcaac
1021 aaactgatgt atacccgtct gctgaaaggt gaagatatca ccctgttcag cccgtccgac
1081 gtaccggggc tgtacgacgc gttcttcgcc gatcaggaag agtttgaacg tctgtatacc
1141 aaatatgaga aagacgacag catccgcaag cagcgtgtga aagccgttga gctgttctcg
1201 ctgatgatgc aggaacgtgc gtctaccggt cgtatctata ttcagaacgt tgaccactgc
1261 aatacccata gcccgtttga tccggccatc gcgccagtgc gtcagtctaa cctgtgcctg
1321 gagatagccc tgccgaccaa accgctgaac gacgtcaacg acgagaacgg tgaaatcgcg
```

FIG. 1A

```
1381 ctgtgtacgc tgtctgcttt caacctggac gcaattaata acctggatga actggaagag
1441 ctggcaattc tggcggttcg tgcactgtac gcgctgctgg attatcagga ttacccgatc
1501 ccggccgcca aacgtggagc gatggatcgt cgtacgctgg gtattgtgt gatcaacttc
1561 gctactacac tggcgcacga cggtaaacgc tactccgacg gcagcgccaa caacctgacg
1621 ataaaacct tcgaagccat tcagtattac ctgctgaaag cctctaatga gctggcgaaa
1681 gagcaaggcg cgtgcccgtg gtttaacgaa accacttacg cgaaagggat cctgccgatc
1741 gatacctata agaaagatct ggataccatc gctaatgagc cgctgcatta cgactgggaa
1801 gctctgcgtg agtcatcaa aacgcacggt ctgcgtaact ccacgctttc tgctctgatg
1861 ccgtccggga cttcttcgca gatctctaac gccactaacg gtattgaacc gcgcgcggt
1921 tacgtcagca tcaaagcgtc gaagacggt attttgcgcc aggtggtgcc ggactacggg
1981 cacctgacg acgctatga gctgctgg gaaatgccgg gtaacgatgg ttatctgcaa
2041 ctggtgggta tcatgcagaa atttatcgat cagtcgatct ctgccaacac caactacgat
2101 ccgtcacgct tcccgtcagg aaaagtgccg atgcagcagt tgctgaagga cctgctcacc
2161 gcctacaaat tcgggtcaa aacactgtat tatcaggacc cccgtgacgg cgctgaagac
2221 gcacaagacg atctggtgcc gtcaatccag gacgatggct gcgaaagcgg cgcatgtaag
2281 atctga
```

FIG. 1B

```
7381 ctgatgccgt caatccagga cgatggctgc gaaagcggcg catgtaagat ctgatatgca
7441 gatgccggat gcggcgtaac cgccttatcc ggcctaccgc tcggtttgta ggcctgatca
7501 gacgcgccag cgtcgcatca ggctccgggt gccggatgca gcgtgaacgc cttatccggc
7561 ctacggctcg gatttgtggg cctgataaga cgcgccagcg tcgcatcagg cacaggatgc
7621 ggcgtaaaat gccttatccg gcattaaact cccaaccagg cacactcatg gcatatacca
7681 cctttcacca gacgaaaaat gatcagctca aggaccgat gttctttggt cagcggtca
7741 acgtggctcg ctacgatcag caaaatatg acatcttcga aagctgatc gaaagcagc
7801 tctctttctt ctggcgtccg gaggaagttg acgtcccg cgaccgtata gattccagg
7861 cgctgccgga gcacgaaaaa cacatcttta tcagcaacct gaaatatcag acgtgctgg
7921 attccattca gggtcgtagc ccgaacgtgg cgctatgcc gcttatttct attcggaac
7981 tggaaacctg ggtcgaaacc tgggcgttct cagaaacgat tcatttccgt tcctatctc
8041 atatcattcg taatatcgtt aacgatccgt ctgttgtgtt tgacgatatc gtcaccaacg
8101 agcagatcca gaaacgtgcg gaagggatct ccagctatta cgatgagctg atcgaatga
8161 ccagctactg gcatctgctg ggcgaaggta cccaaccgt taacggtaaa actgtgaccg
8221 ttagcctgcg cgagtgaag aaaaactgt atctctgcct gatggcgtt aacgcgctgg
8281 aagcgattcg tttctacgtc agcttgctt gttccttcgc attgcagga cgcgaattga
8341 tggaaggcaa cgccaaaatt attgcctga ttgccgcga cgaagcctg cacctgaccg
```

FIG. 2A

8401 gcacccagca tatgctgaat ctgctgcgca gcggcgcgga cgatcctgag atggcggaaa
8461 ttgccgaaga gtgtaagcag gagtgctatg acctgttgt tcaggcagct caacgggaga
8521 aagactgggc ggattatctg ttccgcgacg gttcgatgat tgatctgaat aaagacattc
8581 tctgccagta cgttgaatac atcaccaata tccgtatgca ggcagtcggt ttggatctgc
8641 cgttccagac gcgtccaaac ccgatcccgt ggatcaacac ttggctggtg tctgataacg
8701 tgcaggttgc tccgcaggaa gtggaagtca gttcttatct ggtcgggcag atgactcgg
8761 aagtggacac cgacgatttg agtaacttcc agctctgatg gcccggtta cctgcgcat
8821 cactggcaca caactgctgt gccaggatga acacccttcc cttctgcgg cgctggatc
8881 ccacaatgtg gcggttgagt accagtgtcg cgaaggttac tgcggctcct gtcgcacacg

FIG. 2B

```
301 gtgaacgtcg atctgtgtgc ggatgcagcg gatacgctcc gggcgcaagg atttcgtcaa
361 ttaccggtgg tgatggcggg cgattgagc tggtcgact tccgcccgga catgattaac
421 cgtctgcacc cgaccccca cgcggcaaac gcatggcgc gctgctctac ttctcagcca
481 gctctgaaaa tacgcaccgc tttatgcagc gtctgggct gcctgccacg cgtattccgc
541 tcaatgagcg gggcggattt caggtacgcg aaccgtccat tctggttgtg ccgtcatacg
601 gcggcggcgg gatggccggt gcggtgccgc gacaggtgat ccgcttttta aatgatgaac
661 acaaccggac gcgcattcgc ggcgttatcg cctccggtaa tcgcaattic ggcgatgcct
721 gggatgcgc tggcgatgtg atagcacaaa aatgcggcgt cccctggctg taccgcttig
781 agctcatggg cacacaacgc gacatcgata atgtccgaaa aggagtaaat gaattttggc
841 aacaactacc ccggagcgcg taatgcagga aaccatggat taccacgccc tgaacgcgat
901 gctgaatctt tacgataaag caggccatat tcagttcgac aaggaccagc aggcgatcga
961 cgcctctttt gccacccacg tccgcccgca ttccgtgacg tttgccagcc agcatgaacg
1021 tctgggacg ctggttcggg aggggtatta cgatgcgcc gtcctcgcgc gttacgacg
1081 cgcctcgtc cttcgcctgt tcgagcacgc ccatgccagc ggctttcgct tccagcgtt
1141 tcttggcgcc tggaagttct atcccagtta cacgctgaa acctcgacg gcaaacgtta
1201 tctggaacac tttgaagatc gggtgccat ggtggcgttg acgctggcgc agggtgacga
1261 aacgctggcc acccaactga ccgatgaaat gctttctggt cgctttcagc ccgtacccc
1321 gacttttta aattgcggcc aacagcagcg tgggaactg gtctcctgct tcctgctccg
1381 tatcgaagac aacatggagt cgatcgggcg ggcgttgaat tcggcgctgc aactctcaa
1441 acgcggcggc ggcgtcgcgt ttttcctc caatctgcgc gaggcggcg cgccgatcaa
1501 acgattgag aatcagtctt ccggcgtgat cccggtgatg aaaatgctgg aagacgcgtt
1561 ttcgtatgcc aaccaattg gcgcgcgcca ggggaccggc gcggtttatc tccatgcgca
1621 ccatccggat attctgcgtt ttctggatac caacgagga aacgctgacg aaaaatccg
```

FIG. 3A

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1681 gatcaaaacg ctctctctcg gcgtggtgat cccggatatc acctccggcc tggcgaaaga
1741 aaacgcgcaa atggcgctct tttgacccta tgacataaaa cgaegctacg gcaaaccgtt
1801 tggcgatatc gcaattagcg aacggtacga tgaattaatt gccgatccgc acgtgcgcaa
1861 aacctatatt aacgcccgtg actttttcaa aacactggcg gagattcagt tcgaatcagg
1921 gtatccctac atcatgttcg aagatacggt aaacccgcgc aatcccaatg ctggtcgcat
1981 taatatgagc aacctgtgct cagaatttt acaggtcaat agcgcttccc gttacgacga
2041 taacttgac tatacccaca tcgggcatga catctctgc aatctcggct cgctgaatat
2101 cgctcacgtc atggattcac cggacatigg ccgtaccgta gaaacccgta ttcgcggact
2161 gacggcggtg tcggacatga gccatatacg caggtgccc tcaatagccg ccggtaatgc
2221 cgcctctcat gccatcggtc tgggccagat gaatctgcat ggctatctgg cgaggaagg
2281 tatigcctac ggttcgcgg aggcgttgga tttcaccaat ctctattttt acaccattac
2341 ctggcatgcc gtgcatactt ccatgcggct agcccgcgaa cgcggcaaaa ccttcgccgg
2401 attgcgcag tcgcgctatg ccagcggcga ctatttacg cagtatttac aggcgactg
2461 gcaaccgaaa acagcgaaag tcaggcgct atttgcccgc agcggcatta cgctgccac
2521 acgagaatg tggctaaagc tgcggcgcga tgtgatgcgc tatggcatct atacccaaaa
2581 ttigcaggcg gtgcgccga ccggttcgat ttcttacatt aatcatgcga cctccagcat
2641 tcatccgatt gtggccaaaa ttgagattcg caaggggcc aaaaccgggc gtgtgtatta
2701 cccgcgccg tttatgacca atgaaacct ggacatgtat caggatgctt acgatatcgg
2761 tccggaaaaa attattgata cctatgcgg ggccacgcgc cacgtcgatc aagggctgtc
2821 gctcacctg ttttcccgg atacgccac gacccgcgat atcaaccagg cgcagatcta
2881 tgcctggcga aaagtatta agtcctgta ttacatccgg cttgccagt tggcgtgga
2941 aggtactgaa attgaaggct gcgtatctg cgcgctataa ggaagccat atgaattat
3001 ctcgtattag cgccatcaac tggaacaagg tccaggacga caaagtctg gaggtatgga
```

FIG. 3B

3061 accggctgac cagtaactic tggctgccgg aaaagtgcc gttatcgaat gatattccgg
3121 cctggcggac gctgagcgcc gccgaacagc agctcaccat tcgcgtgttt acggcactia
3181 cgctgctcga cactatccag aacatcgcag gcgcgccgtc gttaatggca gatgccatca
3241 cgccgcatga agggcagtig ctgtcgaaca tcagcttiat ggaagcggta caagcccgct
3301 cttacgttic tattttctcc acgctgtgcc agacgaaaga ggttgatgcc gcctacgcct
3361 ggagcgaaga aaccccaccg cttcagcgta aggcgcagat tattttagct cattacgtca
3421 gcgtgaacc gctaaggaaa aagattgcca gcgtcttttt agagtcittt ctgttctatt
3481 ccggcttctg gttgccgatg tatttctcca gccgcggtaa gctcacgaac actgccgacc
3541 tgattcgttt aatcattcgc gatgaagcgg ttcacggtta ttatattggc tataggtatc
3601 agatagcgct acaaaacta tcggcaatcg agcgtgaaga gttaaagctt ttcgcgctgg
3661 attgttgat ggaacigtac gacaacgaaa tccgctacac agaagcgtta tatgcggaaa
3721 ccggctgggt taacgacgtc aaagcctict tgtgctacaa cgccaataaa gcctiatgia
3781 acctggtta tgaggcgtta tttccgccgg agatggcaga cgtgaatccc gcaatcctig
3841 ccgcgctcic gcgaatgcc gacgaaacc atgatttctt ticcggctca ggttcatctt
3901 atgtgatggg gaaaacgtc gaaccgaag acgaagactg gaattttiaa ccttacgggc
3961 atggaaata acgttacatt tcccatgcct ttatttcaag caataggagg tcaaatcgcg
4021 caatattac aacatgtct acactcaata cgagtgacat tattcactig gattcccca
4081 attcaggtgg attttgctg gttgttccaa aaatatctc ttctcccca ttcgctica
4141 gccctatat catgggaai cacggccgat agcactcgc aatattcatg ccagaggcaa
4201 attcgggtt gtctcgatt ctggtatgt tagggtagaa aaaggtaact atttctatca
4261 ggtaccatat cgaccatagt aaatacagg aatcattcia ttgcatggca attaattag
4321 aagtgaagga tctgtataa atatttgagg agcatccgca gcgtgcttc aaatatatig
4381 aaaaggact atcgaaagag caaatactgg aaaaacggg gctatcgctt ggcgtiaaag

FIG. 3C

```
4441 acgccagtct ggccattgaa gaaggcgaga tattgtcat catggatta tccggctcgg
4501 gtaaatccac aatggtacgc cttctaatc gcctgattga acccacccgc ggacaggtac
4561 tgattgacgg cgttgtatt gccaaatat cagacgctga gcttcgcgag gtgcgcagga
4621 aaaagattgc gatggttttc cagtcatttg cgctcatgcc gcatatgacc gtgctggata
4681 atacggcatt cggtatggaa ttagcgggca tcgcgagcga agagcgtcgc gaaaaagcgc
4741 tggacgcctt gcgtcaggtg gggcttggga attacgctca cgctacccg gatgaacttt
4801 ccggtgggat gcgtcagcgt gttggcttg cccgcgcgct ggcaatcaac cctgatatct
4861 tattaatgga tgaagcgttt tccgccctcg atcc
```

FIG. 3D

```
   1 gaattcttat tttcctagc tttggattta ttctcactic ctatgatctt ttattctcga
  61 ttattatttt tgcttggca attattatca tttttcgaca taaacaaac ctaaaagaa
 121 taaaaatca ttgtgaatcc cttgtcccct ttgattaaa cttatcgaga caaaagaaa
 181 aatagcacaa tatatigtgt tgttttttt ttttttacta attaacact atatctagta
 241 tcttaattt gactagatat ttttttacg ctaaataaga ctataaaac tcgggaaaa
 301 gtcaaggact ttttactccc gtctaaaaa tatattggcc caaaggaga tttaaatgg
 361 ttacagttta ttctaaaaac aattgtatgc aatgcaaaat ggtcaaaaa tggctttctg
 421 aacacgaaat tgcatttaac gaaatcaata ttgatgaaca gcctgaattt gtcgaaaag
 481 taatgaaat gggttttcga gctgctctg taatcacaaa agatgatttc gcttttctg
 541 gtttccgtcc ttctgattta gcaaggttgg cttaatatga aactgctta tttcagtgtg
 601 actggacaaa cgcgtcgttt tgttctcaaa acagactigc cgaatgtcga aattacacct
 661 gacgatgatt tagaggatgg cgagccttic ctttigataa ctccctctta tgctgaggaa
 721 tcaccaaccg tttctaaatc aatagacgtt atggatcgg ttttgactt tatggcttat
 781 aatgataatt ataaacattg tcgtggaatt atcggcactg gaaatcgtaa tttgctggc
 841 atctatattt ttaacgctaa agaagtttca gcaaatatc aaatccact tttatatgat
 901 tttggtttta atggtacgcc agctgatgtt gctgctgttg aaaactcgc tgcacagctt
 961 gatcaaggag cgaaagtcac ctttaaaaat ccgctgtgat ttttatggc ttcacctat
1021 ttgagtgaag ctt
```

FIG. 4

```
   1 cagctgtact ggcataacga catttatact gtcgtataaa attcgactgg
  51 caaatctggc actctctccg gccaggtgaa ccagtcgttt tttttgaat
 101 tttatgaggg ctatgaaaaa cggtgcgaac gctgtttct taagcacttt
 151 tcgcacaaac ttatcttcat tcgtgctgtg gacgcaggc tttatgata
 201 agattgtgc gctaaatacg tttgaatatg atcgggatgg caataacgtg
 251 agtggaatac tgacgcgctg gcgacagttt gataaacgct actctggcc
 301 gcatctctta ttagggatgg ttgcggcgag tttgggttg cctgctca
 351 gcaacgccgc cgaccaaaac gcgcccgcaa aagcgaccac ccgcaaccac
 401 gagccttcag ccaaagttaa ctttgatcaa ttggccttgc tggaagcgaa
 451 cacacgccgc ccgaattcga actattccgt tgattactgg catcaacatg
 501 ccattcgcac ggtaatccgt catctttctt tcgcaatggc acgcaaaca
 551 ctgcccgttg ctgaagaatc ttgcctctt caggcgcaac atcttgcatt
 601 actggatacg ctcagcgcgc tgctgaccca ggaggcacg ccgtctgaaa
 651 agggttatcg catgattat gcgcatttta cccacaagc aaaattcagc
 701 acgcccgtct ggataagcca ggcgcaaggc atccgtgctg gcctcaacg
 751 cctcacctaa caacaataaa ccttcattc atttattaa ctccgcaacg
 801 cgggcgttt gagttttat tatactgatc aagttgtaa ctaagtttt
 851 cgtgatcgt aacgatcgca cctgcgccg gatgcgcgaa gtgatcaaca
 901 tcatcaatgc catgaaccg gagatggaaa aactctcgg cgaagactg
 951 aaggggaaaa ccgcgaagt tcgtgcacgt ctggaggaag gcgaagtgct
1001 ggaaatctg atccagaaag ctttcgccgt gatcatgag gcggtaagc
1051 gcgtctttgg tatgcgtcac ttcgacgttc agttactcgg cgtatatgtt
1101 cttaacgaac gctgcatcgc cggaatgcgt accgatgagg gaaaaccct
```

FIG. 5A

1151 gaccgcaacc ctcctcctt accigaacac actgaccagt gaaggcgtac
1201 acgtgattac cgtcaacgac tacctggcac gacgtgacgc cgaagcaac
1251 catccgctgt ttgattcct tggctgact gtcgtatca acctgccagg
1301 catgccagca ccgcgaaggc gcgaggctta cgcagctgac atcacttacg
1351 gtaccgacaa cgaatcagac tttgactacc tgcacgacaa catgacgttc
1401 agcctgaagg acgtgtacg gcgtgacctg cactatgcac tgatggacga
1451 agtgactcc atcctgatcg atgaagcgcg tacaccactg atcatttccg
1501 gccgacgag gacgactcg gaatgtatg gacgctgag taagattatt
1551 ccgcacctga tcctcaggg aaaagaagac tccgaacct tccgagcga
1601 agcccttc tcgtggacg aaggtctcg ccaggtgacc ctgaccgaac
1651 gtgttctgt gctgattgac gaactgtag tgaaaggagg catcatggat
1701 gaaggggagt ctctgtcctc tccggccgac gtcatgctga tgaccacgt
1751 aacgcggcg ctgcgcctc atgcgtgtt tacccgtgac gtcgactacg
1801 tcgttaagg tgttgagtt atcatcgttg acgacacgc cgttgtacc
1851 atgcaggacc gtcctgatc cgtgatctg caccagctg tgaagcgaa
1901 agaagtgtg cagtccagg acgaagacg gacgtgact tcgatcact
1951 tccgagcta ctccgtctg tatgaaaac tgacaggat gaccgatct
2001 gctgtacgg aggttcga attgctcg gtctacagc tggatacgt
2051 cgttgttcg accgccgtc cgtgattcg taaggctg ccggacctgg
2101 tctacgtgc tgagcgga gagttcagg cgttcattga agatatcaag
2151 gaagtacta cgaagacca gccgtgctg gtggtacta tctccatcg
2201 aggtcgagg ctgtgtcaa acgactgac caaggcggt attgacgag
2251 acgtcctgaa cgccagttc cacgccaacg aggcgacgat tgttgctcag

FIG. 5B

2301 gcagttatc cagctgcgat gactatcgcg gccgatatgg cggtgatgg
2351 tacagatatt gtgtcgatg gtagctgaca ggcggaggtt gccgcgctgg
2401 aggatccgac cgcggcgcaa gttgaggaag ttgaggccga ctggcggtg
2451 cgtcacgatg cagtactgg agcggtggc ctgcatatca tcgtaccgg
2501 gcgtcacgaa tccgtcgta tcgatgacca gttgcggat cgttcggtc
2551 gtcgggggg tgctgttct tccgtttct accigtcgat ggaggtgcg
2601 ctgatgcgtg ttttgctc cgaccggtg tcggcgtgg tgcgtgact
2651 ggatgtggg ccgggcggg ccgtgtgccg cccgtggctg gctgggcgg
2701 ttgccgcgc ccgcgtgcg gtgcgggcc gtgcttcga cgttgtggg
2751 cgctgctgg cgtgtgtgg cgtgctgac gatcgcgtc gcgccgttc
2801 ctccggcgt gacgcctgt tggctgtcg cgtgtggc ggaccgttg
2851 gcgccttcg tgcggtgtg ttcgcggcg ccgttgtgc ctccgttcg
2901 ccgcgtcgc tgcgcggct gtggctgtt ccgggctgc ggggcgtct
2951 gggggccgt ttcgccttc gttccgct tgccggtgg ctggtgggg
3001 gccggcgct gcgtgcggg gcgtcgtg gcgccttct ggcgcgtcc
3051 gtcgggtgt gtcgcgtgc gggcgggtg gttgtgctg ggtgtgtcg
3101 tgcttgggg cgggcgtcg tgtgcgggc gcttgcctcc ctgtgggcg
3151 ggcgctgcc gggtggcgc tgtctgcgc gggtgtccg cctgcgtgc
3201 tgcgccggg cggtccggg gcgggcttc gggcgtggt cgttctccgt
3251 gttgcggcg gtgctgggt cgttgggtg tgggtgtgc ggtcgctgg
3301 gcgggttcg ggtcgtgtg cctgggggg ttgggggct gggccgccg
3351 cgtgtgtgg cggccgggg ttgcgcgcg gtgcgcggc ttgccgtcg
3401 ggtgcggc tctgcgccg cggtgcct gggcgcgcg gcgggggc

FIG. 5C

```
3451 gcaaagtagg acgtaacgat ccttgcccgt gcgattctag tagagaatac
3501 agacatgcc atgcccgcct gcaataaaag ctaactgttg aagtaaagg
3551 cgcaggattc tgcgcctttt ttatggttt aagcaatga aaaagctgca
3601 aattgcggta ggtattattc gcaacgagaa caatgaaatc tttataacgc
3651 gtcgcgcagc agatgcgcac atggcgata aactggagtt tcccggcggt
3701 aaaattgaaa tgggtgaaac gccggaacag gcggtggtgc gtgaacttca
3751 ggaagaagtc gggattaccc ccaacattt ttgctattt gaaaaactgg
3801 atatgaatt c
```

FIG. 5D

```
   1 gatctacggc agaactcgtc gcttggagcg ttcgaccgac catctacctg
  51 ttcgacgtcg aactgcacca ctgaacgtaa tcgccgccag cgcaagtcct
 101 gtcagcgcgt ggcgatcacc gcgcgtgggc gaggccgagt ggtgcggggt
 151 gggcctgcg ccgacggctt ctatgcgcg ctlgaatcag cggtcgtcaa
 201 actggaggc gtgcgccgcg gtaaggatcg ccgcaaggtg cactacggcg
 251 acaaaaccc ggttcgctg gccgggcga ccgcgtggt gccgcgccg
 301 gagaccgct tcaaccccag accgccgag gcaccacgatc acgacggtgc
 351 cgtcgtcgag cggagcctg ggcggatcgt tcgcaccaaa gaacaccgg
 401 ccaagccgat gtcggtcgat gacgcgctct accaatgga gctggttgga
 451 cacgacttct tcttgttcta cgacaaggac accgaccggc cgtcggtggt
 501 ctaccgccgg cacgctacg actacggctt gatccgtctg gcgtgatcgg
 551 cggcgcgcgc cgctcgtcac ctaccatggg agtcgcctta tctaaagact
 601 cctacgatg cgcggacatc gctgtactgt cgaagttgct gcgccttggc
 651 gaagtcgca tggtcaagcg cctcaagaag gtggcggact atgtcgcgac
 701 ttgtccgac gatgtcgaga aactcaccga cgccggcgtg agggcgaaaa
 751 ccgacgagtt cadgcggcgg ctggccgacc agaaaaaccc agaaaacctc
 801 gacgacctgt tgccaggagc cttcgccgtg gcccgcgagg ccgcctggcg
 851 gatgctggac cagcggccgt tcgacgtgca ggtgatgggt gcggccgccc
 901 tgcacctggg cacacgtgcc gggatgaagg ccggtgaagg caagaccctg
 951 acctgtgtgt tgccgcgtta cctcaatgcg ctggcggca acgggcgtgc
1001 catgtcacc gtcaacgact acctggctaa acgcgacgt gagtggatgg
1051 gcgcgtgca ccgcttcctc gggcttcagg tcgggtgat ttcgccacc
1101 atgacacccg atgaacgccg ggtggcctat aacgccgaca tcacctacgg
```

FIG. 6A

```
1151 caccaataac gagttggat tcgactacct gcgcgacaac atggcgcact
1201 cactggatga tctgtgtcag cgcggcacc attacgccat tgtcgacgag
1251 gtcgatttca tcctgatcga cgaggcccgc acccgctga tcatctccgg
1301 tccgccgac ggcctccaac tgctacaccg agttcgccgg ttggcgccgc
1351 tgatggaaaa ggacgtccac tacgagctcg atctacgcaa acgcaccgtc
1401 ggcgtgcacg agaaggtgt ggaattcgtc gaagaccagc tcggcatcga
1451 caacctgtac gaggccgcca actcgccgtt ggtcgctat ctcaacaacg
1501 ctctgaaggc caaggagtcg ttcgccgcg acaaggacta catcgtcgc
1551 gatggtgagg tgctcatcgt cgacgagttc accgccggg tgctgatcgg
1601 ccgccgctac aacgagggca tcgaccaggc catcgaggcc aaggagcacg
1651 tcgagatcaa ggccgaaac cagacgctgg ccaccatcac gctgcagaac
1701 tacttccggc tctacgacaa gctcgccggc atgaccgaca ccgcccagac
1751 ggaggcggcc gagctgcacg agatctacaa gatggcgtg gtcagcatcc
1801 cgaccaacat gcggtgatc cgtgaagacc agtccgacct gatctacaag
1851 acgagaggag ccaagtacat cgcggtggtc gacgacgtcg ccgagcgcta
1901 cgcgaaggga cagccggtgc tgatcggcac caccagcgtg gagcgctcgg
1951 agtatctgtc gcggcagttc accaagcggc gcatcccgca caatgtgctc
2001 aacgccaagt accacgagca agaggcgac atcatcgcgg tggcgggccg
2051 ccgcggcggc gtcaccgtcg ccaccacact ggccgtgtgc ggcaccgaca
2101 ttgtgctggg cagcaacgtc gacttctca ccgatcagcg gctgcgcgaa
2151 cgacctggat ccggtgagga cgcccgagga gtacgaggcg gcctggcact
2201 ccgaactgcc catcgtcaaa gaggaagcca gcaaggaggc caaggaagta
2251 atcgaggccg gcggtgtac gtgctgggca ccgagcggcc acgagtgcg
```

FIG. 6B

```
2301 gcggatcgac aaccagttgc gtggcggtc cggcgccag gggaccccgg
2351 ggagtcgcgc ttctattgt cgtggatga cgagtgatg cgccgttca
2401 atgcgcggc cttggaggacc ttgttgacca ggctgaacct gccgcgcgac
2451 gtgccgatcg aagccaagat ggtcacccgg gccatcaaga gcgcccagac
2501 ccaggtgag cagcggaact ttgggtccg caagacgtc ctcaagtacg
2551 acgggtgat gaacccgccg cgcaaggtca ttacgccga gcgccggcgc
2601 atcctcgaag gcgaaaacct cagggaccag gcgctggcca tggtcgcgga
2651 tgtcatcacc gcctccgtcg acggcgcgac cgccgaggac tatgccgaag
2701 attggatct ggacgcgttg tggcggcac tcaaaccct ctatcgggg
2751 ggatcaccg ccgctcgct gaccgccag gaccacgat tcgagcgcga
2801 cgatctacc cgcggggagt tgtggggc actactcaag gacgcgaac
2851 gtgcctatgc cgccagggga gccgactcg aggaatcgc cggcgaggt
2901 gcgatgcgcc agctggaccg cacgtgctg ctcaacgtca tgaccgtaa
2951 gtggctgaa cacctctccg agatggacta cctcaaggag ggtatcgggc
3001 tgcgcgcgat ggcgccccgc gatccgttgg tcgagtacca gcgtgggggc
3051 tacgacatgt tcatggccat gctcgacggc atgaaaggag aatcggtcgg
3101 cttcctgttc aacgtcaccg tggaggcggt cccgccccg ccgttgccc
3151 cggtgccga acccgcagag cttgccgaat tcgccgccgc ggccgcagcc
3201 gcgggcagca acgcggcgcg gtcgatggtg gcgcgcgca aggctcca
3251 agtgcattac gcgccaggg tgttgccagc gagtcgccg ctttgacta
3301 ttccggtccc gcggggatg gctggctca gatgcaggc aacggcggtg
3351 gagccacca gccgccggcc ggagtgccgg ccggtgctag ccggcggag
3401 cggcgcgaac gcgccgccg acaggccgc ggcgccaggc cgccgaatc
```

FIG. 6C

```
3451 ggtcaagaag cgttagcgcg taggttgcag atggatgtat cgattctca
3501 gttcccagaa gtcactccc ggcccccccc ggcccggcg cgcatgcaca
3551 tttcgttgca cggcggcaa gggattcgct aatctcccc gttgtgcac
3601 ctcgtcggc gtcgttctg ctggtagcgg ggttcggcgc tttcctggcg
3651 tttctcgact cgacaatcgt caacatcgcg ttcccggata tccagcgttc
3701 cttcccgtcc tacgcatcg ggagcctgtc ctgattctg aacggctata
3751 acatgctctt cgccgcattc atggttgcgg ccggcaggtt ggccgatttg
3801 ctgggcgca gacgcattc ctgtccggtg tgctggtgtt caccattgcg
3851 tccgggctgt gcgccgtcgc cggcagttgc gagcagttgg tggcgttccg
3901 ggtgctgcag ggcatcgggg ctgcgatact cgtgcctgat tcgctcgcac
3951 tggtcgttga gggcttcgac cgggcgccg cgcgcacgct atggcctgt
4001 ggggtgcggc ggcacgatc cactggttct agagcggcgc acgc
```

FIG. 6D

```
   1 tcaaacacca gaccagaagg aggcaacacg atacggacg gtgccgttcg
  51 tcggcgggga gcctgggcg gatcgttcgc accaaagaac aaccggcca
 101 cgccgatgtc ggtcgatgac gcgctctacc agatggagct ggttggcaac
 151 gacttcttct tgttctacga caaggacacc gaacggccat cggtggtcta
 201 ccgccggcac gcctacgact acggcttgat ccgtctggcg tcatcggcgg
 251 cgcgccgcgc gtcgtcacct accatgggag tcgccttatc taaggactcc
 301 tacacatgcg gggcatagc tgtgctgtcg agttgctgc gccttggcga
 351 aggtcgcatg gtcaagcgcc tcaagaaggt ggcgactat gtcggcactt
 401 tgtccgacga tgtcgggaaa ctcacgagcg ccgagctgag ggcgaaaacc
 451 gacgagttca agcaggctgg ccgaccagaa aaacccagaa accttgacg
 501 acctgttgcc cgaggcttc accgtgcccc gcgagcccg cctgccggct
 551 gctggaccaa cgcccgttcg acgtgcaggt gatgggtacg acgcccttgc
 601 acctgggcga cgttgccgag atgtagaccg gtgaaggcaa gaccctgacc
 651 tgtgttttac ccgcttacct caatgcctg gcgccaacg gcgtgacgt
 701 agttaccgtc aacgctacc tggctaacg cgacagtgag tggatgggcc
 751 gcgtgcaccg cttcctcggg cttcaggtcg ggtgatttt ggccaccatg
 801 acacccgatg aacgcgggt ggcctatacc gccgacatca cctacggcac
 851 caataacgag tttggttcg actacctgcg cgacaacatg gcgcactcac
 901 tggatgatct ggtgcagcgc gggcaccatt acgccattgt cgacgaggt
 951 cgattccatc ctgatcgacg agggcgggac ccccccccca tctccgcccg
```

FIG. 7A

```
1001 gggcgcccgc ctccaactgg ttcaccgggt tcgaccggtt ggcgtgccgc
1051 ggctggtttt ggacgtccac tacgagtcg atctacgcaa acgcacgtc
1101 ggcgtgcacg agaggggtgt ggattcgtc gaagaccagc tcggcatcga
1151 caacctgtac gagaccgcca actcgccgtt ggtcagctat ctcaacaacg
1201 ctctgaaggc caaggactg ttcggcgcg acaaggacta catcgtccgc
1251 gatggtgagg tgctcatcgt cgacgattc accggccggg tgctgatcgg
1301 ccgccgatac aacgaggga tgaccaggc catcgaggcc aaggagcacg
1351 tcgaggatca ggccgagaac cagacgctgg ccaccatcac gctgcagaac
1401 tacttccggc tctaggagaa gctgccggg atg
```

FIG. 7B

```
   1 tggcttgatt caaactagtg aacaataaat taagtttaaa gcacttgtgt
  51 ttttgcacaa gttttttat actcaaaaag caaattatga ctatttcata
 101 gttcgataat gtaattgtt gaatgaaaca tagtgactat gctaatgtta
 151 atggatgtat atattgaat gttagttaa taatgtatg teagtctat
 201 gtatagtccg agtcgaaaat cgtaaaatat ttataatata atttattagg
 251 aagtataatt gcgtattgag aatatattta ttagtgataa actgttgac
 301 aacagaatgt gaatgaagta tgtcataaat atattatat tgattctaca
 351 aatggtaaa taagtataat tttctaacta taaatgataa gatatatgt
 401 tgtaggccaa acagttttt agctaaagga gcgaacgaaa tgggattttt
 451 atcaaaaatt cttgatggca atagtaagga aattaaacag ttagtaaac
 501 ttgctgataa agtaatcgct ttagaagaaa aacggcaat tttactgat
 551 gaagaattc gtaataaaac gaaacaattc caacagaat tagctgacat
 601 tgataatgtc aaaagcaaa atgattattt acataaaatt ttaccagaag
 651 catatgcact tgttaggga ggctctaaac gtgtattcaa tatgacacca
 701 tataagttc aaattatggg tggtattgca attcataaag gtgatatcgc
 751 tgagatgaga acaggtgaag gtaaacatt aacagcgaca atgccaacat
 801 acttaaatgc attgctggt agaggtgttc acgttattac agtcaatgaa
 851 tacttatcaa gtgttcaaag tgaagaatg gctgagttat ataacttctt
 901 agtttgact gtcggattaa acttaaacag taagacgaca gaggaaaaac
 951 gtgaagcata cgcacagac attacttaca gtactataa tgagctagt
1001 ttgattact tacagatta catggtgat tattctgaag atagggtaat
1051 gcgtccatta catttgcaa tcattgatga ggtggactca attttaatcg
```

FIG. 8A

```
1101 acgaggcacg tacgccatta attatttctg gtgaagctga aaagtcaacg
1151 tcacttttta cacaagcaaa tgttttgcg aaaatgttaa aacaggacga
1201 tgattataaa tacgatgaca aaacgaaagc tgtacattta acaggaacaag
1251 gtgcggataa agctgcacgt atgttcaaag ttgaaaactt atatgatgta
1301 caaaatgttg atgttattag tcatatcaac acagcittac gtgcgcacgt
1351 tacattacaa cgtgacgtag actatatggt tgttgatggc gtagtattaa
1401 ttgtcgatca atttacagga cgtacaatgc caggcgtcg tttctggaa
1451 ggtttcaacc aagttattga agcgaaggaa ggcgttcaaa ttcaaaatga
1501 atctaaaact atggcgtcta ttacattcca aaactatttc agaatgtaca
1551 ataaacttgc gggtatgaca ggtacagcta aaactgaaga agaagaattt
1601 agaatatttt atacatgac agtaactcaa attcgacaa atgaaccigt
1651 gcaacgtaac gataagtctg atttaattta cattagccaa aaagtaaat
1701 ttgatgcagt agtagaagct gttgttgaaa aacacaagc agggcaacca
1751 gtgctattag gtacigttgc agttggact tctgatata tttcaaattt
1801 acttaaaaaa cgtggtatcc gtcatgatgt gttaactgcg aaaatcatg
1851 aacgtgaagc tgaaattgtt gcaggcgctg gacaaaagg tgccgttact
1901 attgccacta acatggctgg tcgggtaca gatatcaaat taggtgaagg
1951 cgtagaggga ttaggcggtt tagcagtaat agglacgag cgacatgaat
2001 ctcgtcgtat tgatgaccag ttacgtggtc gttctggacg tcaaggtgat
2051 aaaggggata gtcgcttcta tttatcatta caagtgat taatgattcg
2101 ttttggttct gaacgtttac agaaaatgat gagccgacta ggtttagatg
2151 actctacacc aattgaatca aaaatggtat caagagctgt tgaatcagca
```

FIG. 8B

```
2201 caaaaacgtg tagaaggtaa taacttcgac gcgcgtaaac gtatcttaga
2251 atacgatgaa gtattacgta aacaacgtga aattatctat aacgaaagaa
2301 atagtattat tgatgaagaa gacagctctc aagttgtaga tgcaatgcta
2351 cgtcaacgat tacaacgtag tatcaattac tatattaata cagcagatga
2401 cgagcctgaa tatcaaccat tcatcgacta cattaatgac atctcttac
2451 aagaaggtga cattacagag gatgatatca aaggtaaaga tgctgaagat
2501 attttcgaag tcgttgggc taagattgaa gcagcatatc aaagtcaaaa
2551 agatatctta gaagaacaaa tgaatgagtt tgagcgtatg atttacttc
2601 gttctattga tagccattgg actgatcata tcgacaacat ggatcaatta
2651 cgtcaaggta ttcacttacg ttcttatgca caccaaatc cattacgtga
2701 ctatcaaaat gaaggtcatg aattattga tatcatgatg caaaatattg
2751 aagaagatac ttgtaaattc atttaaaat ctgtagtaca agtgaagat
2801 aatattgaac gtgaaaaaac aacagagttt ggtgaagcga agcacgtttc
2851 agctgaagat ggtaagaaaa aagtgaacc gaaaccatc gttaaggcg
2901 atcaagttgg tcgtaacgat gattgtcat gtggtagtgg taaaaattc
2951 aaaattgcc atggaaata aatgatataa aatactcct tccaattaaa
3001 cacctatagt ttgtgttatg ggaggagtct tttatttta caagcgttaa
3051 atacttttaa aaatgtgaag aagttgttaa acgttgttat gtacttagtt
3101 ttaaaaatc ggttaggca tatg
```

FIG. 8C

```
   1 cttgaagtt aattactaa tgtgacgaat gtgaatgcac atgtaaaagt
  51 gaaacttat gcaaatctc gcacaaaatc gaagttacaa ttcgcttaa
 101 tgacgtgca cttgtgcag aggaaggaa cgatgattta tgctggaatt
 151 gacaagatca ctaccaaatt cgaatgtcaa gttcgtaact ccaaaacacg
 201 tgtcaatcgt aagcacgta aagaaagcga acatgaccca ttcccagcaa
 251 ctcggaaac tcgcacggaa acagctgttg atcatgatca agatgatgaa
 301 attgaatca tcgttctaa acaattcagc ttgaaaccaa tggattctga
 351 agaagcggta ttaccaatgg atttacttgg tactgatttc ttcatcttca
 401 atgaccatga aactgatggt acaagcattg tttaccgccg taaggacgga
 451 aatatggtt tgattgaaac tgtgaaaaa ctaatatgtg atatttgaaa
 501 ggctcttgc tgcatttttct gctgcaggag tttcttttt tgaggaagcc
 551 cttattaaga tttgattaat aaaaatacca ttgattgatt tacacggggt
 601 gtccatgtca aaataagagg gatgtattaa gttcattatt gtatgtgag
 651 ctcgatgag tgagcggcat atgattatga tatccatgtg gcacatgatg
 701 ttaacaaaaa gagaatgaca ctgtgagaag tacatcttga taaacacaac
 751 taggcagttt attaaaaat aatgaacagt atccttgag tttttaagta
 801 taattaagc catataaatg gtaagataaa ttgttgtaag ccaaacagtt
 851 tttataccaa aggagcgaac agaatggatt ttttaacaaa aattgttgac
 901 ggcaataaga gagaatcaa acgcctaagt aagcaagctg acaaagtaat
 951 ctcattagga gaagaatgt caattcttac tgatgaagaa attagaaata
1001 aaacaaaagc attccagaga agattgcaag cagaagaaca tgtaagcaaa
1051 caagataaaa tttagaaaga aatattacct gcagcatttg cgcttgtccg
1101 tgaaggagct aaacgtgtat ttaatatgac acttatcca gttcaatca
1151 tggtggtat cgccattcat aatggtgaca tttcagaaat gaggacaggt
```

FIG. 9A

```
1201 gaagataaaa cattaactgc aacgatgccg actatttaa acgcttagc
1251 agcacgtggt gtgcatgtta ttacgtcaa tgatactttg gcaagttctc
1301 aaagggagga aatgacggag ttatataatt tcattggttt atcgtcgga
1351 ttgacttga acagctatc aacgaacaa aagcgtgaag cttatcatgc
1401 agtattacg tatgtacaa atattgaatt aggcttcgac tatttacgcg
1451 atacatggt gaatattca gaagaacgtg ttatgcgtcc gcttcatttc
1501 gctatcattg atgagtcga ctctatttta atcgatgaag cgcgtacacc
1551 attgattatt tcagggaag ctgaaaaatc aacatcttt tatacaaag
1601 caaigtttt cgctaaaatg ttaaaagcag aagtgatta taattatgat
1651 gaaaaaaca aatcagtaca attacagat caaggtgctg ataagctga
1701 acgtatgttc aagttagata actatatga tttgaaaaac gttgatatta
1751 tcacgcatat caatacagca ttacgtgcta actatacatt gcaacgcgat
1801 gtagattaca tggttgtgga tggaaagta ttgattgtcg accaatttac
1851 aggtcgaaca atgccaggtc gtcgattctc tgaaggactt cacccagcga
1901 ttgaggctaa agaaggggtt caaattcaaa atgatctaa aacaatggct
1951 tctatcacat tccaaaacta cttcgtatg tatataaat tagcggtat
2001 gacaggtact gctaaaacag aggaagaaga attccgtaac attatcata
2051 tgacgttac acaattcca acgaccgtc ctgttcacg tgaagatga
2101 ctgactga tttcatcag ccaaaaggc aagtcgatg ctgttgtga
2151 agatgtgtt gaaaacata aaaaggcca accaattct ttaggtactg
2201 tagcggtga aacaggtaa tacatttcac aactatgaa aaaacgagt
2251 gtgcgtcatg atgtcttaa cgctaaaac catgaacgcg aagctgaaat
2301 cgtatctaca gcaggtcaaa aggtgcagt cacaatcgca acaacatgg
2351 ctggtcgtgg tacgatatt aaattaggcg aaggtgtga agattaggc
2401 gacttgctg ttatggtac agaacgtat gaatcacgcc gtatcgatga
```

FIG. 9B

```
2451 tcagttgcgt ggtcgttctg gacgacaagg tgacgcgcgg gaagccgttt
2501 tctattatac attccaagat gagttgatgg tacgttccgg ttctgaacgt
2551 ctgcaaaaaa tgatgggccg attggtatg gatgactcta cacgattga
2601 atcaaaatg gtatctcgag ctgttgaatc tgccaaaaaa cgtgttgaag
2651 gtaccaactt cgatgcacgt aaacgtatct tagcatacga tgaagtttta
2701 cgtaaacaac gtgaaatcat ttatgttgaa cgtaatcata ttatcgattc
2751 agaatcaagt tctgaattag tcattacaat gatacgctct ccattgatc
2801 gtgcaatcag ttattatgta aatgaagaat tggaagaaat tgactatgcg
2851 ccgtttatta atttgtgga agatgtttc ttdcacgaag gtgaagtcaa
2901 agaagatgaa atcaaagta aagtaaaga tcgtgaggat atttcgata
2951 cagtatggac taaaattgaa aaagctatg aagcacaaaa agccaatata
3001 ccegaccaat tcaatgaatt cgaacgtatg atttattac gttctattga
3051 tggaagatgg acagccata tcgatacaat ggatcaatta cgtcaaggta
3101 tccattacg ttcatacggt caacaaacc cacttcgcga ctatcaaaat
3151 gaaggcaac aactattga tacaatgatg gtcaatatg aagaagacgt
3201 cagcaatat atcttgaaat caattatcac agtgagtgat gatattgaac
3251 gtgataaagc aaagaatat caaggacaac atgtatcagc tgaagatgga
3301 aaggaaaag taaaccgca accagttgtt aaagataatc acatcggaag
3351 aatgatcct tgtccatgcg gcagcggtaa aagtatcaa aatgctgcg
3401 gtaatgtgta agttgtatta ggaccactgt taaatagctt taaggagat
3451 gctcaattga aatggatta tcttctaag ggctgtcagc ggtcttttt
3501 caatccaaca aaaatatgga tatatgctaa aataatgag taatctggaa
3551 aattaactg gaattggag gatatgaaaa tggaattat
```

FIG. 9C

```
   1 cagtcaatgt cgctctcgt gaccgagcca atggacggaa aggtgcccgc ctcccagatc
  61 atgaacctcc tagtgtcagc ctatcagaag ggccttaaga cgggcctcta ctactgcaag
 121 atccgcaagg ccaccaccaa cggctcttc acggcgagcg acctcgtgtg ctctgggtgc
 181 cacctgtagc gacgcgcgcc gtgcgagccg cggacgcggc gaccctcacg
 241 cgtaaatacc aatactttta cgagcccgag tgcccgacc tagatcactt gcggtcgctc
 301 agcgtcgcaa acgctggct ggagcccgag tttcccctag cggacgacgc caggggcgtg
 361 gcgcggctca gcggcgccga gtcggagttt taccgcttc tgttcgcgat cctctcggcc
 421 gccgtgacc tcgtgacgt caacctcggg gacctgtccg agctgtccaa ccaaaaggac
 481 atcctgcatt actatatcgc gcaggatcc atcgaagtgg tgcactcgcg ggtgtacagc
 541 gccatcagc tgctgctct tggaacgac gcggtgcgc gcgcggcta cgtggaggc
 601 gccctcggcg acccgcgat cggcgcaag gtggactggc tcgagcggcg cgtggccgcg
 661 gcagatcgg tggccgaaaa gtacgtgctc atgattctaa tcgaggcat ttttttctcc
 721 tcctcgttta cggcgattgc ctactcgac acccaaacc tttcgtcgt gacgtgccaa
 781 accaagacc tcatcagccg cgccgagcc gtgcaccgg ccgctcgtg ctgcattcc
 841 gacaactacc tcggcgggga gcggccgccg ccggccgcca tctacgagct gttccgcgaa
 901 gcgtggaaat tgagcgcgag tttattggt tgcgccgcc gcggcagtca tatcatgac
 961 gtggaggcta tttctgcgta cgtcgagtac agcgcggacc gcctgctcgc tgctatcaag
1021 ctgcctctc tgtttgcac ccgcctcct gggaccgatt ttcctttggc cctgatgact
1081 gccgagagc acacgaactt ctttgagcgc gcagcacca actacacagg caccgtgatc
1141 aacgacctgt agggcacccc cgctgccctg ccaggcgcc ccgctttcc tcctcttct
1201 cacccccacg ccgcgatac aaaatgttcc atgtcacgg aa
```

FIG. 10

```
   1 tcgagcccgc cgaaacccgc cgcgtctgtt gaaatgacca gccgcccdgc cgcatcctct
  61 ccgtcgaag cgcggcccc ggttgggga caggagccg gcggccccag cgcagccacc
 121 cagggggagg ccgccgggc ccctcigcc cacgccacc acgtgtactg ccggcggtc
 181 aatggcgtga tggtgctttc cgacaagacg ccccggtccg cgtcctaccg catcagcgat
 241 agcaccttg tccaatgtgg ttccaactgc accatgatca tgacggagga cgtggtgcgc
 301 gggcgcccc aggaccccgg ggccgcggca tccccgctc ccttcgtgc ggtgaccaac
 361 atcggagccg gcagcgacgg cgggaccgcc gtcgtggcat tcgggggaac cccacgtcgc
 421 tcggcgggga cgtctaccgg taccccagcg gccgacgtcc ccaccgaggc ccttgggggc
 481 cccctcctc ctcccgctt caacctgggt ggcggctgtt gttcctgtcg cgaccacagg
 541 cgccgtctg cggtatccgg ggggagggag gatccagtcg gcccgcggga gttcgtctcg
 601 gacgaccggt cgtccgattc cgactcggat gactcggagg accaggactc ggggacgctg
 661 tcaccagcct cctggacgt gtccggcggg gccaccgtacg acgacgccct tgactccgat
 721 tcgtcatcgg atgactccct gcagatggat ggcccagtgt gtcgcccgtg gagcaatgac
 781 accgcgcccc tggtgtttg ccccgggacc cccggccggg gcgccgacgc cggtggtccc
 841 tcagcggtgg accccaccgc gccgacgcca gaggccggcg ctggtcttgc ggccgatccc
 901 gccgtggccc gggaagacgc ggaggggctt tcggaccccc ggccacgtct gggaacgggc
 961 acggctacc ccgtcccct ggactcacg cccgagacg cggaggccgt ggcgcgcttt
1021 ctgggagatg ccgtgaaccg cgaacccgcg ctcgtgctgg agtactttg ccggtgcgcc
1081 cgcgaggaaa ccaagcgtgt ccccccaagg acattcggca gccccctcg cctcacggag
1141 gacgacttg ggcttctcaa ctacgcgctc gtggagtgc agcgcctgtg tctggacgtt
1201 cctcggtcc cgccgacgc atacatgccc tattatctca gggagtatgt gacgcgctg
1261 gtcaacggt tcaagccgct ggtgaccgg tccgctcgcc ttcacgcat cctgggggtt
1321 ctggtgcacc tgcggatccg gacccgggag gcctcctttg aggagtggct gcgatccaag
```

FIG. 11A

```
1381 gaagtggccc tggattttgg cctgacggga aggcttcgcg agcacgaggc ccagctggtg
1441 atcctggccc aggctctgga ccattacgac tgtctgatcc acagcacacc gcaacacgtg
1501 gtcgagcggg ggcgcatc ggccctgagg tatgaggagt tttacctaaa gcgttttggc
1561 ggcactaca tggagtccgt cttccagatg tacaaccgca tgccgaggct ttggcctgc
1621 cgggccacgc gcggcatgcg ccacatcgcc ctgggcgag agggctcgtg gtgggaaatg
1681 ttcaagttct ttttccaccg cctctacgac aaccagatcg tacgtcgcac ccccgacatg
1741 ctgaacctgg ggacccgcaa ctactacacc tccagctgct acttggtaaa ccccaggcc
1801 accacaaaca agcgacctt gcgggccatc acagcaacg tcagtgccat cctgaccgc
1861 aacgggggca tcggctatg cgtgcgggcg tttaacgact ccggcccagg gacgcaagc
1921 gtcatgccg ccctaggat ccttgactcg ctggtgggg cgcacaacaa ggagggcgcg
1981 cgtccgaccg gcgcgtgcgt gtacctggag ccgtggcaca ccgacgtgcg ggccgtgctc
2041 cggatgaagg gggtcctgc cggcgaagag gccacgctgt gcggacatat ctcaggcgac
2101 ctctggatgc cagacctgtt tttcaagcgc ctgattcgcc acctgaacgg cgaggaggac
2161 gtcacatgga ccctgttcga ccgggacacc agcatgtcgc tcgccgactt tcacgggggag
2221 gagttcgaga agctctacca gcacctcgag gtcatgggat tcggcgagca gatacccatc
2281 caggagctgg cctatggcat tgtgcgcagt gcggccacga ccgggagccc cttgtcatg
2341 ttcaagacg cggtgaaccg ccactacatc tacgcaccc agggggcggc catcgccggc
2401 tccaacctct gcaccgagat cgtccatccg gcctccaagc gatccagtgg ggtctgcaac
2461 ctggaagcg tgatctggc ccgatgcgtc tccggcaga cgtttgactt tgggcggctc
2521 cgcgacgccg tgcaggcgtg cgtgctgatg gtgaacatca tgatcgacag cacgtacaa
2581 cccacgccc agtgcaccg cggcaacgac aacctgcgat ccatgggat cggcatgcag
2641 ggcctgcca cggctgcct gaggctgggg ctggatctg agtctgcga atttcaggac
2701 ctgacaaac acatgccga ggtgatgctg ctgtcggcga tgaagccag caacgcgtg
```

FIG. 11B

```
2761 tgcgttcgcg gggcccgtcc cttcaaccac tttaagcgca gcatgtatcg cgccggccgc
2821 tttcactggg agcgctttcc ggacgcccag ccgcgtacg aggcgagtg ggggatgcta
2881 cgccggagca tgatgaacca cggctgcgc aacagccagt ttgtcgcgct gatgccaacc
2941 gccgcctcgg cgcagatctc ggcgtcagc gagggcttg ccccctgtt caccaacctg
3001 ttcagcaggg tgacccggga cggcggacg ctgcgcccca acacgctcct gctaagggaa
3061 ctggaacgca cgtttagcgg gaagcgcctc ctggaggtga tggacagtct cgacgccaag
3121 cagtggtccg tgccgcggc gctcccgtgc ctggagccca ccacccccct ccggcgattc
3181 aagaccgcgt ttgctacga ccagaagttg ctgatcgacc tgtgtgcgga ccgcgccccc
3241 tacgtcgacc atagccaatc catgaccctg tatgtacgg agaaggcgga cgggacctc
3301 ccagcctcca ccctggtccg ccttctggtc cacgcatata agcgcggact aaaaacgggg
3361 atgtactact gcaaggttcg caaggcgacc aacgcgggg tcttggcgg cgacgcaac
3421 atigtctgca tgagtgcgc gctgtgaccg acaaacccc tccgcgccag gcccgcgcc
3481 actgtcgtcg ccgtccccag ctctcccctg ctgccatg
```

FIG. 11C

```
   1 gtgtgtttgg cgtgtgtctc tgaaatggcg gaaacccaca tgcaaatggg attcatggac
  61 acgttcaccc ccctgactc aggagatagg catatctctc ttagattgac tcagcacacg
 121 atcgcacccc acccctgtgt gcgggggata aaagccaacg cgcgcggtct gggttaccac
 181 aacagtggg tgcttcgggg acttgacggt cgccactctc ctgcgagccc tcacgtcttc
 241 gcccaccgat tcctgtgcg ttcctgtcgg ccggtgctgt cctgtgaca gattgtggc
 301 gactgcccgg gtgattcgtc ggccggtgcg tcctttcggt cgtaccgccc acccgcctc
 361 ccacgggccc gcagtgttt ccgtcatcg cgtccgagcc acgtcacct tggttccaat
 421 ggccaacgc cctgccgcat ccgcctcgc cgggcgcgg tctccgtccg aacgacggga
 481 acccgggag cccgggttcg ccccccttgg cggcaccac gtgttttgca ggaagtcag
 541 cggcgtgatg gtgcttccg gcgatcccc cggcccgcg gcctaccgca ttagcgccag
 601 cagctttgtt caatgcggct ccaactgcag tatgatgatc gacggggacg tggcgcgcgg
 661 tcattgcgt gacctcgggg gcgctacgtc caccggcgcc ttcgtcgcga tctcaaacgt
 721 cgcagccggc gggatggacc gaacgccgt cgtggcgctc ggcggaacct cgggccgtc
 781 cgcgactacg tccgtgggga cccagcgctc cgggggattc ctccacggga acccaggac
 841 cccgaaccc caaggaccc aggctgtcc cccgcccct cctccccct ttccatgggg
 901 ccacgagtgc tgcgcccgtc gcgatgccag gggcggcgcc gagaaggacg tcgggccgc
 961 ggagtcatgg tcagacggcc cgtgtccga ctccgaacg gaggcatggg actcctcgga
1021 cgaggatacg ggctcgggtt cgggacgct gtctcgatcc tcttcgatct gggccgcgag
1081 ggcgactgac gacgatgacg gcgactccga ctcgcggtcg gacgactccg tgcagcccga
1141 cgtgtgctt cgtcgcagat gggcgcgacgg ccctgcccc gtggcttttc ccaagccccg
1201 gcgccccggc gactccccg gaaccccgg cctgggcgcc ggcacgggc cgggctccgc
1261 gacggacccg cgcggtcgg ccgactccga ttccgcggcc cacgcgccg cacccaggc
1321 ggacgtggcg ccggttctgg acagccagcc cactgtggga acggacccg gtacccagt
```

FIG. 12A

```
1381 cccccagaa ctcacgcccg agaacgcgga ggcggtggcg cggttctgg gggacgccgt
1441 cgacgcggg cccgcgctca tgctggagta ctctgttgg tgcgccgcg aggbggacaa
1501 gcgcgtgccc ccacgaacct tcggcgcgc ccccgcctc acggaggacg acttgggct
1561 cctgaactac gcgctcgtg agtgcgacg cctgtgcctg gacctccccc cggtcccccc
1621 caacgcatac acgccctatc atctgaggga gtatgcgacg cggctggtta acggttcaa
1681 acccctggtg cggcgtccg ccgcctgta tcgcatcctg gggatctgg ttcacctgcg
1741 catcgtacc cgggaggcct ccttgaggg atggatgcgc tccaaggagg tggacctgga
1801 cttcgggctg acggaaggc ttcgcgaacg cgaggccacg ctaatgatcc tggcccaggc
1861 cctgaacccc tacgactgtc tgatccacag cacccgaac acgctgtcg agcgggggct
1921 gcagtcggcg ctgaagtacg aggatttta cctcaagcgc ttcggcgggc actacatgga
1981 gtccgtcttc cagatgtacg cccgcatcgc cgggttcctg gcatgccggg cgacccgcgg
2041 catgcgccac atcgccctgg ggcgacaggg gtcgtggtgg gaaatgttca agttcttttt
2101 ccaccgcctc tacgaccacc agatcgtgcc gtccacccgc gccatgtga acctggaac
2161 ccgcaactac tacgatccg gctgatacct ggtaaaccc caggcacaca ctaacgagc
2221 cacctccgg gccatcaccg gcaacgtgag cgccatcctc gcccgcaacg gaggcatcgg
2281 gctgtgcatg cgggcgttca acgacgccag cccgggaccc gccagcatca tgccggccct
2341 gaggtcctg gactcctgg tggcgggcga caacaaacag agcacgcgcc ccaccgggac
2401 gtgcgtgtac ctggaaccct ggcacagcga cgttcggcc gtgctcgga tgaaggcgt
2461 cctcgccggc gaggaggccc agcgctgcga caacatcttc agcgccctct ggatgaccga
2521 cctgttcttc aagcgcctga tccgccacct cgacggcgag aaaacgtca cctggtccct
2581 gttcgaccgg gacaccagca tgtcgctcgc cgactttcac ggcgaggagt tcgagaagct
2641 gtacgagcac ctcgaggcca tgaggttcgg cgaacgatc ccatccagg acctggcgta
2701 cgccatgtg cgcagcgcgg ccaccaccgg aagcccctic atcatgttta aggacgcggt
```

FIG. 12B

2761 aaacagccac tacatctacg acacgcaagg ggcggccatt gccggctcca acctctgcac
2821 ggagatcgtc caccgctcct ccaaacgctc cagcgggctc tgcaacctgg gcagcgtgaa
2881 tctggcccga tgcgtctccc ggcggacgtt cgattttgac atgctccgcg acgccgtgca
2941 ggcgtgcgtg ctaatggtta atatcatgat ggacagacag ctgcagccga cgccccagtg
3001 cgcccgcggc cacgcaaccc tgcggtccat gggcattggc atgcagggcc tgcaacgggc
3061 gtgcctgaag atggcctgg atctggagtc ggcggagttc cggacctga acaacacat
3121 cgccgaggtg atgctgctcg cggccatgaa gaccagtacc gcgctgtgcg ttcgcggggc
3181 gcgtcccttc agccacttta agcgcggcat gtacccggcc ggccgcttc actgggagcg
3241 ctttcgaac gccagcccgc ggtacgaggg cgagtgggag atgctacgcc agagcatgat
3301 gaacacggc ctgcgcaaca gccagttcat cgcgctcatg cccacgacg cctggcca
3361 gatctggac gtcagccagg gctttgccc cctgttcacc aacctgttca gcaaggtgac
3421 cagggacggc gagacgctgc gcccaacac gctcttgctg aaggactcg agcgacgtt
3481 cggcgggag cggctcctgg acgcgatgga cgggctcgag gccaagcagt ggtctgtggc
3541 ccaggcctg cctgccatgg acccgcgcca cccctccgg cgattcaagg cggcttcga
3601 ctacgaccag gaactgctga tcgacctgtg tgcagaccgc gcccctatg ttgatcacag
3661 ccaatccatg actctgtatg tcaccaggaa ggcggacggg acgctcccg cctcaacct
3721 ggtccgcctt ctcgtccacg catataagcg cggctgaag acggggatgt actactgcaa
3781 ggttcgcaag gcgaccacca gcggggtgtt cgccggcgac gacaactcg ttgacgcaag
3841 ctgcgcgctg tcagcaaccg cgctcgatc ggggtcaggc gtcgctctcg gtccgcata
3901 tcgccatgga tcccgccgtc tcccccgcga gcaccgaccc cctgatacc cacgctcgg
3961 ggccgcgggc ggcccgatt ccggtgtgcc ccacccccga gcggtacttc tacacctccc
4021 agtgcccga catcaaccac cttcgtccc tcagcatcct gaacgctgg ctgaggaccg
4081 agctcgtgtt cgtcgggcac gaggaggacg tctccaagct ctccgagggc gagctcggct

FIG. 12C

```
4141 tctaccgctt tctgttgcc ttcctgtcgg ccgcggacga cctggtgacg gaagacctgg
4201 gcggctctc cggcctcttc gaacgaagg acattcttca ctactacgtg gagcaggaat
4261 gcatcgaggt cgtccactcc cgcgtctaca acatcatcca gctggtgctc tttcacaaca
4321 acgaccagge gcgccgcgcc tatgtggccc gcaccatcaa ccaccggcac attgcgtca
4381 aggtggactg gctggaggcg cgggtgcggg aatgcgactc gatcccgaag aagttcatcc
4441 tcatgatcct catcgagggc gtctttttg ccgcctcgtt cgccgccatc gcgtacctgc
4501 gcaccaacaa cctcctgcgg gtcacctgcc agtcgacgga cctcatcagc cgccacgagg
4561 ccgtgcatac gacgcctcg tgtacatct acaacaactg cctcgggggc caagccaagc
4621 ccgggcggc gcgcgtgtac cggctgttcc gggaggcgat ggatatcgag atcgggttca
4681 tccgatccca ggccccgacg gacagctcta tcctgagtcc gggggcctg gcggccatcg
4741 agaactacgt gcgattcagc gcggatcgcc tgctgggcct gatccatatg cagcccctgt
4801 attccgcccc cgccccagac gccagtttc ccctcgcct catgtccacc gacaaacaca
4861 ccaacttctt cgagtgccgc agcacctcgt acgccgggac cgtcgtcaac gatctgtgag
4921 gatctgggcg ccctgtggc gatgtctaac cgaaataaag gggtcgaaac ggactgttgg
4981 gtctcggtg tgattattac gcggggaagg gggtggcgg ctgggaaag ggaaggaacg
5041 ccgaaccca gagaaagga ccaaaggga aacgcgtcca acgataaat caagccgca
5101 ccggaccct gggatgcata ataccaacg atttattac tcttattatt aacgggtcgg
5161 gcatcgggg gggatgggg cgcgcgttc ctcgttccg gctactgtc ccagaattc
5221 gccaggacgt cctgtaaaa cgcgggcggg ggcgcgtggg ccccacctg cgccagaaac
5281 cgttcggcga tgtcgggggc ggtgtatga cgagtcacga tgggcgcgc taatcttcg
5341 tcgcgggggt cctgatagat gggcagtctt tttgaggagg tccaggtcc ccgctcttg
5401 ggctgataa gcgatatgac gtacttgacg tatctgtgct ccacccgctc ggcgatggtc
5461 atcggatcgg gcagccagtc cagggcctcc ggggcgtcgt ggatgacgtg gcggcgacgt
```

FIG. 12D

```
5521 ccggcgacat agccgcggtg ttccgcgacc cgctgcgcgt tgggacctg  cacgagctcg
5581 gacgggctga gtatctccga ggaggacgac cgggcgccgt cagcgggccc  acgggcgacg
5641 tccgggggct gggggggggg gtattttctg tagtcgtact cgccgcgat  ctgttggcc
5701 agaatttcag tccacgagat gcgcgtctcg aggccgaccg gggcgcggt  cagcgtgggc
5761 atgctccca gggagcgcga gttggcggac tccgccgggg ccgccgggg  ggcctgggat
5821 cgcctcgggg cggtccagtg acactcgcgc agcacgtcct cgacggacgc  gtagctgtta
5881 ttgggtgca ggtctgtgtg gcagcggacg aacagcgcca ggaactgcgg  gtaactcatc
5941 ttgaagtacc ctgcag
```

FIG. 12E

```
   1 aaaccactgt tctttacact ttatgctcta gttttggta atagtgtctt ggaacacttt
  61 tacctaaac gaaatatgg cttggattt tttgagcacc gactgtccac tggggatgt
 121 ttcgatatt atatccaacg tgaatacaat caaaggtat ggattttca gcgatttatc
 181 aacaacgtg gcaactcgcc cgtctcggga acagtgtta gagtatatca ccaggtcgt
 241 ggataaatc aagcgctgt gcagagtcga cgaacgcctt tacattgcgt gcgggagct
 301 tgtacacta cgaataaag cacgcaacac agcctgaaa tattggctaa aatcgtctga
 361 gattgatctt agcgatgtcg tggaacaggc catattggaa cacattgact ttgttaggaa
 421 aacctcaac tcgttgaaa catcggatta acgagattg tgttcatag gcctgcaatc
 481 tgcgctaaag tatgaggaaa tgtatttagc caaaatgcga ggcggacgtc tagagtccat
 541 ggggcaattt tttcttagac ttgcaactac tgctacgcac tatactatgg aacaaccagc
 601 aatggctgc gtgttggtta gcggtgaggt tggctggaca tatatttca gagcttttt
 661 tactgcgcta gcggacagg ttgtcattcc ggccacgcca attatgctgt ttggtgggag
 721 agactgtggg tctatggcca gctgttattt gctaaaccc agggtaacag atatgactc
 781 tgcaattccg gctcttatgg aggagttgg acccatttg tgcaaccgag gaggaattgg
 841 actgtcttta cagcgttta acactccacc cacagaggt tgttcacggg gtgtcatggc
 901 tctcctaag ctactagct ctatgccat ggccattaac agcgacggtg aaggaccaac
 961 aggagtgtgt gttattttcg aacctgcca cgcagacatc cgcgccattt taaatatgcg
1021 cggatgctg gccgagacg aaactgtgcg ctgcgacaac atcttgctt gtatgtggac
1081 cccgacctg tttttgacc gctatcaacg gtacgtgat ggagaagcg gcataatgtg
1141 gactctgtt gatgatactg catcgacct ctgccatatg tacggaatg attcacacg
1201 ggaatatgag cgcctggagc ggtgtggatt tggaatagac gctattccca tacaggcat
1261 ggccttatc atagttagaa gtgctgtaat gacgggaagc ccatttttga tgtttaaaga
1321 cgcgtgcaac aggcactacc acttgacat gcggcagagg ggtgcgatac tggggtctac
```

FIG. 13A

```
1381 tctatgccca gaaattatcc agcatgccga cgaaacccaa aacggggtgt gtaatctagc
1441 cagcatcaac ctcccaaagt gtctagcctt tccacctcca aatattgcag gtgtgccata
1501 ttttgacttc gcagctctgg gccgcgctgc cgccaatgcc acaatttttg tcaatgcgat
1561 gatgtgtgcc agcacatatc caactgttaa atcccgagaa ggcgttgaag aaaacgggtc
1621 gctgggactt ggaattcagg ggctccatac cacgttttcg atgctggacc tggatatggc
1681 atctccagag gcgcacccaa ttaaccaggca aatagcagga aggctgttat tgaactctat
1741 gaaggccagc gcaacgctct gcaagctggg tatgcaaccc tttaaaggat ttgaagacag
1801 caagtacagt cgggggaaac taccctttga tgcctaccca aatgtaacac taacaaacag
1861 caacgcctgg cgtagattc gcactgacat aaaacaatac ggctgtacca attctcagtt
1921 tgtgcctat atgccaccag tatcttcgtc acaggttacc ggagcaggcg agggtttttc
1981 tcctgtttac acaaactgt ttagcaaggt tactgctacc gggaagtac tcaggcccaa
2041 tgtactgcta atgcgcacca tcagaagtat ttttccaccg gaatgcgcgc gcttacaagc
2101 gctatctacg ctagaagctg cgaatggctc agttgtgggg gcgtttggtg attgccagt
2161 tggtcccccc ctcagtaagt ttaaacagc atttgagtac gaccgactg tgctaattaa
2221 catgtgtgct gacgggggtg cgtttgtgga ccaggccaa tccatgtctt tgtttatcac
2281 tgagcctgct gacggaaaac tcccagcctc cagaattatg aatctttgg tccacgcata
2341 taaacgcgga cttaaaacag gcatgtacta ctgccaaatc aggaggcaa caaacaccgg
2401 agtctttgtt ggcggagacc tagtctgcac cagtgcagc ttgtgggca gcctgccat
2461 tttgcccagg gcgggaaaat aattatggcc ctcgaaact ctaaaaaac agatttgct
2521 gacgagttat tgataaatgc gtatttctat acgccggaat gtccgatat tgaacacctg
2581 cgcttgttga gcgttgccaa ccgctggctg gatacggacc ttccaatttc tgatgacctc
2641 aaggacgttg ctaaactcgc gccagccgag cgaggatttt accggttttt gtttgccttt
```

FIG. 13B

2701 ttatctgctg ctgacgactt ggtaaattta aacctgggag atttatcagc actatttact
2761 caaaaggaca ttcttcacta ctacattgag caggagtcta ttgaaglaac gcactccaga
2821 gtatatagcg ctatacagct tatgttgttt ggaaacgacg caacagcgcg cgctaggtat
2881 gtcgcatctg ttgtcaaaga cgtggccata gacctaaagg tatcttggtt gcaagcaaag
2941 gtgcgaggat gcaaatctgt ggcggaaaag tatatttgta tgatattaat agaggcgttt
3001 ttcttcgcgt cgtctttcc gtccatcgca tatcttcgca cccacaatct ctttgtggta
3061 acctgtcaaa gtaatgattt aattagccgc gacgaagcaa ttcacaccaa cgcctgtgc
3121 tgtatctaca acaactacct tgggcgtttt gaaaagccag ctccaacgag gatttatgcg
3181 ctgttttctg aggccgtaaa catcgagtgt gatttttgc tttccatgc cccaaaagc
3241 agccacctgt tggacattga agccatcata tgctacgtac gctatagcgc ggacaggctt
3301 ttgggggaaa ttggactatc tccgctgttt aatgctccca aacccccacc aagcttcccc
3361 ctagctttca tgactgtgga aaaccatacc aacttttttg aagggcgaag cacgcatac
3421 tcgggaactc ttataaagca tctgtatgt aaaaataaaa actatttttg attcacttat
3481 ttgtcttgtt tgcgtgttgg atgtacgcga tttaaaaaa tactggaaa agatactcc
3541 gatttaactt tatttaagac catttgtctc ggtgtccaca gtcatccag tagtaacca
3601 acacagtgtt gtatcagtg gggtgggaa tgtggttcca aaacatatta gcaagctctc
3661 tgacaatttc gtgttcgg

FIG. 13C

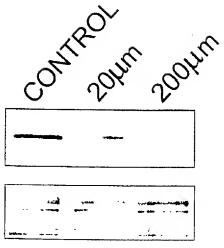

FIG. 14

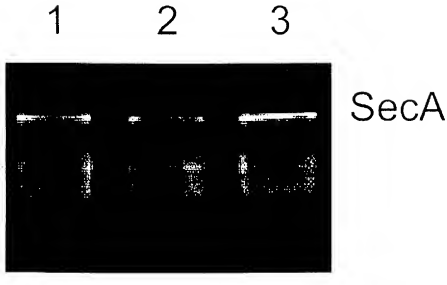

FIG. 17

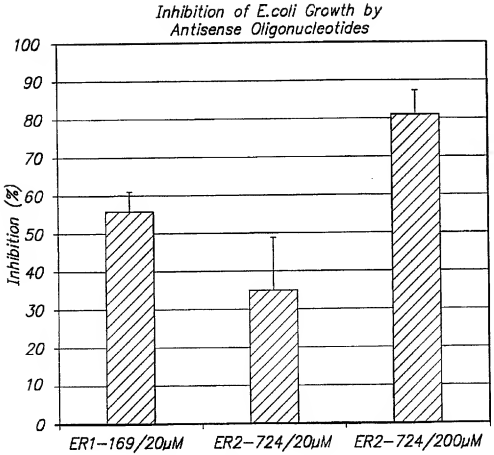
Inhibition of E.coli Growth by Antisense Oligonucleotides
Inhibition (%)
100
90
80
70
60
50
40
30
20
10
0
ER1–169/20μM
ER2–724/20μM
ER2–724/200μM

FIG. 15

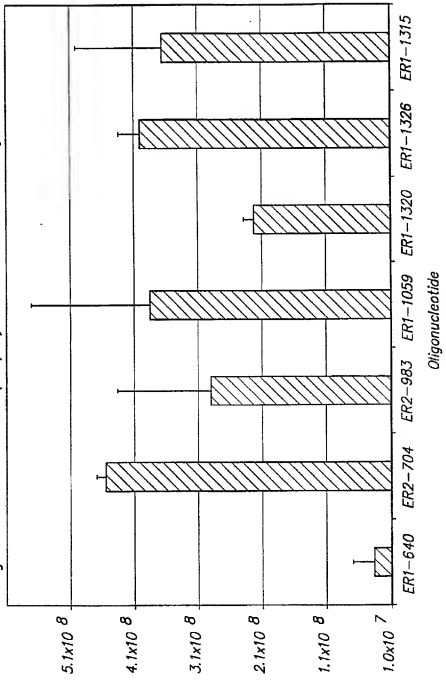
Killing of Escherichia coli K12 (CFU/ml) treated with antisense oligonucleotides (20μM)
CFU/ml
5.1x10 8
4.1x10 8
3.1x10 8
2.1x10 8
1.1x10 8
1.0x10 7
ER1-640
ER2-704
ER2-983
ER1-1059
ER1-1320
ER1-1326
ER1-1315
Oligonucleotide

FIG. 16

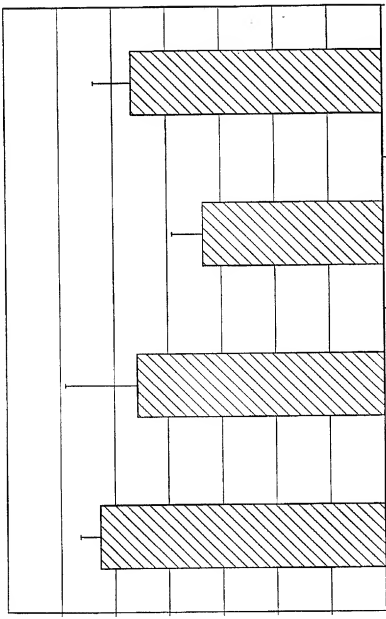
Killing of Escherichia coli K12 (CFU/ml) with antisense oligonucleotides (100µM)
CFU/ml
1.3x10 8
1.1x10 8
9.0x10 7
7.0x10 7
5.0x10 7
3.0x10 7
1.0x10 7
ES129
ES320
ES386
ES556
Oligonucleotide

FIG. 18A

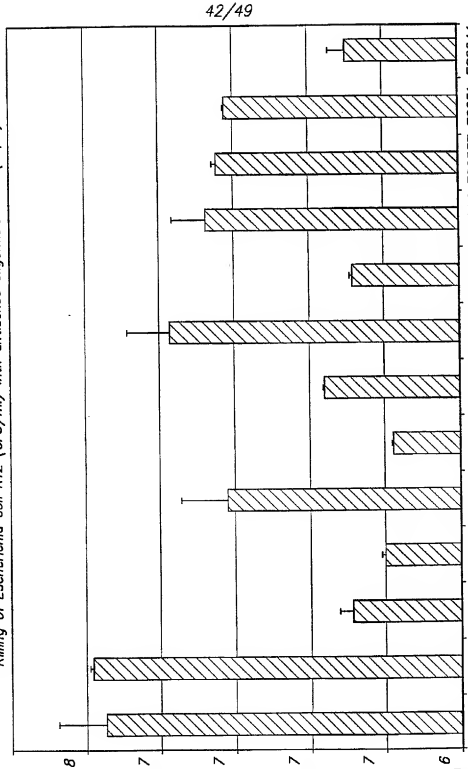
Killing of Escherichia coli K12 (CFU/ml) with antisense oligonucleotides (20μM)
CFU/ml
1.0x10 8
8.1x10 7
6.1x10 7
4.1x10 7
2.1x10 7
1.0x10 6
ES386
ES531
ES647
ES799
ES851
ES1128
ES1398
ES1581
ES1739
ES1845
ES2537
ES851
ES2644
Oligonucleotide

FIG. 18B

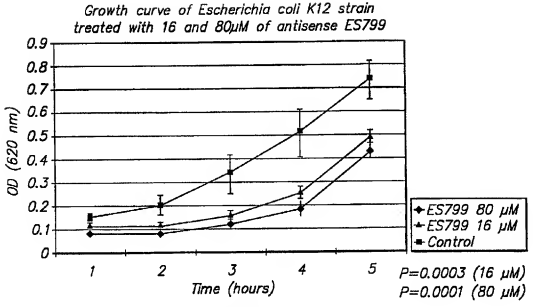
Growth curve of Escherichia coli K12 strain
treated with 16 and 80µM of antisense ES799
OD (620 nm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
Time (hours)
ES799 80 µM
ES799 16 µM
Control
P=0.0003 (16 µM)
P=0.0001 (80 µM)

FIG. 19A

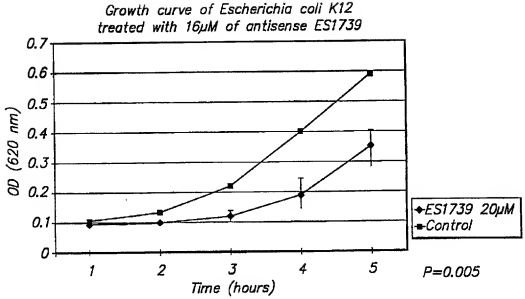
Growth curve of Escherichia coli K12
treated with 16μM of antisense ES1739
OD (620 nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
Time (hours)
ES1739 20μM
Control
P=0.005

FIG. 19B

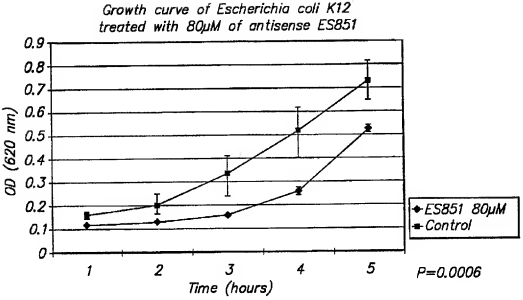
Growth curve of Escherichia coli K12
treated with 80µM of antisense ES851
OD (620 nm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
Time (hours)
ES851 80µM
Control
P=0.0006

FIG. 19C

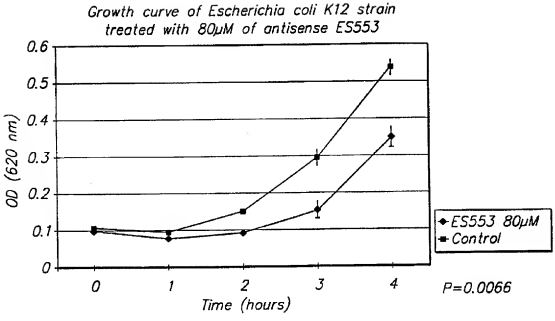
Growth curve of Escherichia coli K12 strain
treated with 80μM of antisense ES553
OD (620 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
Time (hours)
ES553 80μM
Control
P=0.0066

FIG. 19D

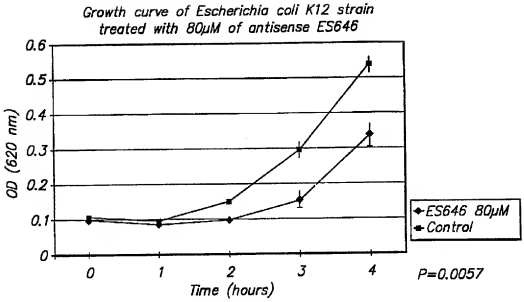
Growth curve of Escherichia coli K12 strain
treated with 80µM of antisense ES646
OD (620 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
Time (hours)
ES646 80µM
Control
P=0.0057

FIG. 19E

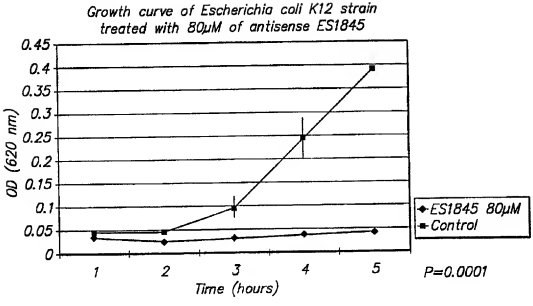
Growth curve of Escherichia coli K12 strain
treated with 80µM of antisense ES1845
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
OD (620 nm)
1
2
3
4
5
Time (hours)
ES1845 80µM
Control
P=0.0001

FIG. 19F

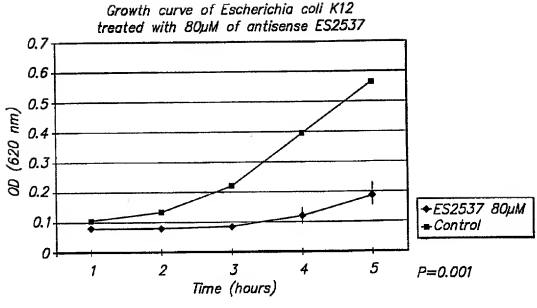
Growth curve of Escherichia coli K12
treated with 80μM of antisense ES2537
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD (620 nm)
1
2
3
4
5
Time (hours)
ES2537 80μM
Control
P=0.001

FIG. 19G